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THE UNIVERSITY OF ALBERTA

The Effect Of Environmental Temperature On Protein Turnover In The Laboratory Rat

by

© Tim M^{c}Allister

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

Animal Biochemistry

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1987

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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled The Effect Of Environmental Temperature On Protein Turnover In The Laboratory Rat submitted by Tim M^{c}Allister in partial fulfilment of the requirements for the degree of Master of SCIENCE in Animal Biochemistry.

J R Thompson

Supervisor

Date August 17/82

ABSTRACT

Experiments were conducted to determine the effect of hot and cold environmental temperatures on protein turnover in several tissues of the laboratory rat. In order to accomplish this task a sensitive automated high-performance liquid chromatographic method was developed for the estimation of phenylalanine as its decarboxylated product, ß-phenylethylamine, in tissue cell fluids or protein hydrolysates. The system is extremely sensitive, enabling the accurate determination of 0.5 pmol ß-phenylethylamine. The necessity of correcting for endogenous ß-phenylethylamine in cell fluid when calculating the fractional rate of protein synthesis in the liver was demonstrated.

A preliminary experiment was conducted to determine the specific radioactivity-time course changes of tissue free phenylalanine in three skeletal muscles and the liver after rats were given an intraperitoneal injection of ^{3}H-phenylalanine. Groups of 20 rats were exposed to temperatures of 5, 25 or 35 C for 10 d. The free phenylalanine specific radioactivity differed significantly (P<0.001) between all tissues. The free phenylalanine specific radioactivity was lower in liver than in skeletal muscle. The free phenylalanine specific radioactivity in the tissues of rats at 35 C was higher (P<0.001) than that of both control rats and rats at 5 C. The fractional rate of protein synthesis in the soleus muscles of rats exposed to 5 C was lower ($P<0.05$) and tended to be lower in rats exposed to 35 C in comparison to rats exposed to 25 C. The fractional rate of protein synthesis in the gastrocnemius muscle tended to be lower in rats exposed to 35 or 5 C when compared to the rate in control rats. Cold and heat exposure tended to increase the rate of protein synthesis in the liver.

A second experiment was performed to determine the effect of environmental temperature on protein turnover in skeletal and heart muscle and liver. Groups of 36 rats were exposed to temperatures of 5, 25, or 35 C for 25 days. In addition, a group of 36 rats was maintained at 25 C and fed amounts equivalent to that consumed by rats in the hot environment. Protein turnover was measured at 5, 10, 15 and 20 days of temperature exposure. Exposure of rats to cold or hot temperatures tended to decrease the absolute rates of both protein synthesis and degradation in skeletal muscle in comparison to control values. Cold exposure increased the absolute rate of protein synthesis ($P<0.05$) and degradation in the heart and liver, while heat exposure tended to cause a decrease in comparison to control rats. Similar trends in the rate of protein turnover in tissues from heat-exposed and pair fed rats suggest that alterations in protein turnover are due, in part, to changes in feed intake.

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I. INTRODUCTION

Schoenheimer et al. (1939) were the first to demonstrate that proteins in the animal body are continuously undergoing degradation and resynthesis. These two processes are collectively termed protein turnover. Protein turnover is an essential process for cell survival and has several purposes. During periods of starvation, amino acids mobilized by protein breakdown are an important energy substrate (Wassner et al. 1977; Garlick et al. 1975). Protein turnover can regulate the flux through metabolic pathways by changing the quantities of rate-limiting enzymes (Schimke 1977). Protein turnover is also required for the removal of abnormal proteins which may cause cell damage (Bradley et al. 1976). Finally, protein turnover is essential during the growth and the restructuring of cells resulting from anatomical changes associated with activities such as exercise (Booth and Watson 1985; Mohan et al. 1985).

The purpose of this introduction is to briefly review the two components of protein turnover, synthesis and degradation. Two of the methods used for measuring protein turnover in vivo will be examined, followed by a brief description of the factors which regulate this process. The introduction will conclude with a review of the known effects of environmental temperature on protein turnover and nitrogen balance in animals and with an outline of the objectives of the present study.

A. PROTEIN SYNTHESIS

1. Initiation

A number of reviews of the initiation of protein synthesis have been published (e.g. Grunberg-Mango and Safer 1982; Moldave 1985). The initiation of protein synthesis requires the presence of ribosome subunits, messenger RNA, initiation factor proteins and the initiator methionyl-tRNA. Initiation begins with the binding of methionyl-tRNA to GTP and the initiation factor protein eIF-2 to form a ternary complex. The ternary complex binds to the 40S ribosomal subunit forming a methionyl-tRNA subunit complex which then binds to the specific initiation codon on the mRNA. This reaction requires ATP and the presence of four initiation factor proteins (eIF1, eIF3, eIF4A, eIF4B). Initiation is completed when a 60S ribosomal subunit becomes associated with the complex in a reaction which is dependent on another initiation protein (eIF5) and the hydrolysis of GTP. The methionine remains bound to the tRNA and in position for the formation of the first peptide bond.

2. Elongation

The processes involved in peptide elongation have been reviewed by several authors (e.g. Mazumder and Szer 1977; Moldave 1985). The first step in peptide elongation is the binding of a second aminoacyl -tRNA to the ribosome in preparation for the covalent bonding of the first two amino acids. This reaction requires the hydrolysis of GTP and the presence of protein elongation factor 1 (eEF-1).

The second stage of peptide elongation is catalyzed by the ribosome and results in the formation of a peptide bond between methionine and the incoming amino acid. With the formation of the peptide bond, the linkage between methionine and tRNA is broken, leaving the dipeptide attached to the ribosome by the second tRNA.

The final stage of peptide elongation is known as translocation. In this step the first tRNA dissociates from the ribosome. The second tRNA which is joined to the dipeptide moves over and occupies the vacated site on the ribosome. Elongation factor 2 (eEF-2) and a second GTP are used to move the ribosome one codon along the mRNA . The aminoacyl-tRNA corresponding to the next mRNA codon can then bind to the ribosome. This cycle continues to add one amino acid at a time until the entire peptide chain is synthesized.

3.Termination

The processes controlling the termination of protein synthesis are not as clearly defined as those of initiation and elongation. It is known that a termination codon dictates where synthesis of the peptide chain will stop. However, unlike the initiation codon, the termination codon does not code for a particular amino acid. A single protein called a release factor (eRF) has been shown to catalyze the termination of protein synthesis by binding to the ribosome (Moldave 1985). During termination the completed peptide chain is released from the tRNA and the tRNA detaches from the ribosome. In the final step of termination the ribosome and mRNA dissociate.

B. INTRACELLULAR PROTEIN DEGRADATION

The processes of intracellular protein degradation are classified into two pathways; nonlysosomal and lysosomal. Both pathways are required for the regulation of cell protein content.

1. Nonlysosomal Degradation Pathway

The nonlysosomal pathway is responsible for the breakdown of short-lived and abnormal proteins (Knowles and Ballard 1976; Amenta and Brocher 1981). The pathway is not sensitive to nutritional deprivation or hormonal action and is responsible for the majority of protein degradation during normal metabolic conditions (Ciechanover et al. 1984). Several types of proteases are involved in nonlysosomal protein degradation. Most recent research has concentrated on the ATP and Ca^{++}-dependent proteases (Hershko and Ciechanover 1982; Pontremoli and Melloni 1986)

As the name implies, ATP-dependent proteolysis requires energy in the form of ATP. Hershko and Ciechanover (1982) proposed that ATP is required for the covalent bonding of the marker protein ubiquitin, to proteins scheduled for degradation. Once ubiquitin is bonded to the protein, proteolytic enzymes selectively recognize and completely degrade the ubiquitin-protein conjugates in the cytosol. The non-lysosomal proteolytic enzymes are not well characterized and most work has concentrated on Ca^{++} dependent proteases.

Demartino and Croall (1985) demonstrated that there are two types of Ca^{++}-dependent proteases, CDP-1 and CDP-11 which become activated at different calcium ion concentrations. These proteases are restricted to partial degradation gf peptides. Ca^{++}-dependent pro eases have been implicated in the breakdown of cytoskeletal (Hatanaka et al. 1984), membrane receptor (McGowan et al. 1983) and muscle myofibrillar proteins (e.g. Pontremoli and Melloni 1986; Sugita et al. 1983;). The exact biochemical mechanisms involved in the degradation of these proteins by Ca^{++}-dependent proteases are unknown but several theories exist (e.g. Pontremoli and Melloni 1985; Melloni et al. 1982).

2. Lysosomal Degradation Pathway

Lysosomes are membrane bound vesicles which contain endopeptidases and exopeptidases capable of degrading cell proteins. To accomplish degradation, the lysosome surrounds a cytoplasmic component containing protein in a process known as autophagy (Saito and Ogawa 1974). Once engulfed, the low pH within the lumen of the autophagic vacuole denatures the protein and peptidases cleave the peptide bonds releasing free amino acids. Unlike the nonlysosomal pathway, lysosomal protein degradation is accelerated by nutritional deprivation and is sensitive to hormonal changes (Ward et al. 1977; Mortimore and Nealy 1975). For example, several studies have demonstrated that thyroid hormones can enhance lysosomal enzyme activity in rat skeletal muscle and liver (De Martino and Goldberg

1978; Millward et al. 1985). The lysosomes are thought to be responsible for the degradation of long-lived proteins under conditions of pathological and nutritional stress (Hershko and Ciechanover 1982). The contribution of the lysosomal pathway to the degradation of long-lived proteins during normal physiological conditions remains uncertain (Poole et al. 1978).

C. MEASUREMENT OF PROTEIN TURNOVER IN VIVO

The estimation of protein turnover in vivo has been attempted through the use of isotopic techniques. Estimations of protein synthesis have been based upon the rate of incorporation of a labeled amino acid into protein from a precursor pool of known specific radioactivity. The specific radioactivity of amino acids in intracellular or extracellular free amino acid pools has been used as a value for the specific radioactivity of the precursor pool in most studies because of problems associated with estimating the specific radioactivity of aminoacyl-tRNA (MacDonald et al. 1979). Protein degradation is then estimated as the difference between protein accretion and protein synthesis (Goldspink et al. 1983; Reeds et al. 1982). The continuous infusion and single flooding dose techniques are the two commonly used methods for establishing a constant steady state specific radioactivity of the precursor amino acid.

1. Continuous Infusion Technique

Earlier researchers (e.g. Lajatha 1957; Roberts and Morelos 1965) used a single dose of a labeled amino acid to determine the rate of protein synthesis. In these experiments several samples were required in order to estimate the specific radioactivity-time curve of the precursor amino acid pool. Therefore, continuous infusion of a labeled amino acid was used in several subsequent studies (Dietz et al. 1982; Lobley et al. 1980; Simon et al. 1978; Garlick et al. 1976) to establish a plateau in the specific radioactivity of the precursor amino acid. In this procedure a labeled amino acid is infused until a plateau in the specific radioactivity-time curve of the free amino acid pool is reached. With the continuous infusion technique, protein synthesis is estimated with only a single measurement of the specific radioactivity of protein bound and free amino acids once a plateau has been reached. This represents a major advantage over earlier single dose techniques in which a number of samples were required to estimate the time course of the specific radioactivity of the precursor amino acid pool (Millward et al. 1976).

Despite these advantages several limitations are inherent in the constant infusion technique. Several hours of infusion may be required (Lobley et al. 1980; Simon et al. 1978) to reach a plateau in the specific radioactivity-time curve. During this period labeled amino acids can be incorporated into protein and subsequently released by degradation into the free amino acid pool. The recycling of labeled amino acids causes the rate of protein synthesis to be underestimated. Errors arising from recycling of labeled amino acids can be reduced by decreasing the length of time the labeled amino acid resides in the tissue (Waterlow et al. 1978). The constant infusion method is also subject to problems in estimating the specific radioactivity of the precursor amino acid at the site of protein synthesis (Garlick 1980a). With the constant infusion technique it is possible that the specific radioactivity of the intracellular and extracellular free amino acid pools differ from each other or from that of the aminoacyl-tRNA pool. As Waterlow et al. (1978) point out, any discrepancies between the specific radioactivity of the assumed precursor pool and aminoacyl-tRNA pool will produce an error in the estimate of protein synthesis. Finally, Preedy and Garlick (1984a) have recently demonstrated that immobilization of an animal during infusion of the labeled amino acid affects the rate of protein synthesis (see section E-2).

2. Single Flooding Dose Technique

The injection of a single very large dose of a labeled amino acid minimizes most of the major problems associated with the constant infusion technique. This method was first used by Loftfield and Eigner in 1958. The principal behind this technique is that the injection of a flooding dose of the labeled amino acid results in the establishment of a similar specific radioactivity in the extracellular, intracellular and aminoacyl-tRNA precursor amino acid pools. With the specific radioactivity in all three pools approaching equality, the specific radioactivity of any of the pools can be used to estimate the specific radioactivity of the precursor pool for protein synthesis. The amount of recycling of labeled amino acid is also reduced since a labeling period of only 10 min is used (M^{c}Nurlan et al. 1979; Garlick et al. 1980a). The single flooding dose also reduces stress as it is only necessary to immobilize the animal during the time of the single injection in contrast to immobilization throughout the constant infusion period.

The most commonly used amino acid for the flooding dose technique is ^{3}H-phenylalanine (Garlick et al. 1980a) since, it readily dissolves in aqueous solution, there is a relatively small free phenylalanine pool, it is relatively inexpensive compared with other labeled amino acids and its specific radioactivity can be relatively easily determined. Furthermore, phenylalanine unlike other amino acids such as leucine, has not been shown to effect protein turnover (Fulks et al. 1975). Garlick et al. (1980a) demonstrated that even the high concentrations of phenylalanine used in the flooding dose technique did not alter the rate of protein synthesis in rat tissues.

The single flooding dose technique is also subject to a few limitations. The procedure is mainly restricted to small animals due to the increased cost of isotope required to flood the large free amino acid pools present in large animals. For this reason, continuous infusion is still the method of choice in large animals. Secondly, the ninhydrin procedure of Garlick et al. (1980a) commonly used to quantitatively determine phenylalanine is time consuming and the derivative is relatively unstable, a problem which is rectified in the present study through the development of a high-performance liquid chromatography method for quatitative analysis of phenylalanine (see chapter II).

E. REGULATION OF PROTEIN TURNOVER

1. Hormones

Of all of the hormones known to influence protein turnover, the effects of insulin have been most thoroughly characterized. Insulin has an anabolic effect on tissues resulting in a net deposition of protein by accelerating amino acid transport into the cell (Narhara and Hollosy 1974), promoting the initiation of RNA translation (Jefferson et al. 1977; Jefferson 1980) and the inhibition of protein degradation (Stirewalt and Low 1983; Pain et al. 1983). Jefferson (1980) demonstrated that insulin accelerates protein synthesis by improving the translational efficiency and by increasing the amount of mRNA and ribosomes. The inhibition of protein degradation by insulin has been suggested to involve an alteration in lysosomal function through a reduction in the formation of autophagic vacuoles (Mortimore and Nealy 1975). It is unclear which chemical mediator(s) act as a secondary messenger(s) to produce these changes in protein turnover after insulin binds to its plasma membrane receptor, but several theories exist. For example, Reeds et al. (1985) suggested that prostaglandin $F_{2\alpha}$ may be the secondary messenger as they have demonstrated that indomethacin, an inhibitor of prostaglandin synthesis, prevented the stimulation of protein synthesis by insulin. Jarret et al. (1982) have proposed that there are two chemical mediators of insulin action, the first responsible for dephosphorylation of enzymes and the second responsible for the phosphorylation of enzymes.

It has been demonstrated that treatment of hypophysectomized rats with growth hormone increases the rate of skeletal muscle protein synthesis (Flaim et al. 1978). Earlier studies demonstrated that growth hormone decreases the rate of protein degradation in skeletal muscle (Bartlett 1955) but more recent studies indicate that growth hormone has no effect on skeletal muscle protein degradation (Flaim et al. 1978; Goldberg and Griffin 1977).

Only a few researchers have examined the effect of glucagon on protein turnover. Beatty et al. (1963) suggested that glucagon decreases muscle protein synthesis in vitro. Preedy et al. (1980) supported this conclusion when they demonstrated that glucagon decreases protein synthesis in the perfused rat hemicorpus. In a recent in vivo study, Preedy and Garlick (1985), have shown that glucagon decreases protein synthesis in skeletal muscle, has no effect on heart muscle and increases protein synthesis in the liver. There appears to be no reports on the effect of glucagon on tissue protein degradation.

Corticosteroids have been shown to decrease skeletal muscle protein synthesis (Kelly and Goldspink 1982; Odedra et al. 1983) and to increase liver and heart protein synthesis (Odedra et al. 1983;

Clark et al. 1986). The decrease in muscle protein synthesis results from reduced amino acid uptake into the myofiber (Kostoyo and Redmond 1966), inhibition of ribosomal RNA synthesis (Bullock et al. 1972) and a reduction in peptide chain initiation (Rannels et al. 1978). Chronic administration of corticosteroids decreases liver protein degradation (Odedra et al. 1983) and increases heart protein degradation (Clark et al. 1986). In the rat, daily administration of catabolic doses of corticosterone results in enhanced skeletal muscle protein degradation for a 4-5 day period (Odedra et al. 1983) after which the rate returns to preadministration values. The effects of corticosteroids on muscle protein breakdown are dependent on a corticosteroid-insulin interaction. In the fed state, higher insulin levels partially inhibit corticosteroid stimulated proteolysis (Millward et al. 1985). In the fasted state, decreased insulin levels and elevated corticosteroid levels accelerate muscle proteolysis. The exact mechanisms responsible for corticosteroid enhanced proteolysis are unknown but non-lysosomal degradation may be involved (Clark and Vignos 1979).

Most researchers that have examined the effect of thyroid hormones on protein turnover have concentrated on the active thyroid hormone, tri-iodothyronine (T_3). In cardiac muscle elevated plasma T_3 concentrations have been shown to cause an increase in protein synthesis and to have no effect on protein degradation (Carter et al. 1982). In skeletal muscle and liver, T_3 produces an increase in both protein synthesis and degradation. In the euthyroid condition the rate of protein synthesis exceeds degradation and protein deposition occurs in the growing animal, while in the hyperthyroid condition protein degradation exceeds synthesis resulting in a loss of protein (Millward et al. 1985). Tri-iodothyronine increases protein synthesis by promoting the translation of mRNA (Brown et al. 1983) and increasing rates of protein initiation and elongation in collaboration with insulin (Millward et al. 1985). The demonstration that T_3 enhances lysosomal enzyme activities in rat skeletal muscle and liver (De Martino and Goldberg 1978; Millward et al. 1985) suggests that T_3 increases protein degradation through the lysosomal pathway.

2. Immobilization

Denervation or immobilization of skeletal muscle results in a rapid rise in proteolysis and a fall in protein synthesis (Goldspink et al. 1983; Goldspink 1977). Preedy and Garlick (1984a) demonstrated that immobilization of the rat for one hour during tail-vein infusion reduced skeletal muscle protein synthesis by 32 %. Watson et al. (1984) suggest that alterations in the initiation of protein synthesis may be responsible for this decline during immobilization. The increased proteolysis in these conditions likely arises from activation of the lysosomal pathway in muscle (Libby and Goldberg 1978).

3. Nutritional state

It has been well documented that short-term fasting inhibits protein synthesis in several body tissues (Emery et al. 1986; Preedy et al. 1984b; M^{c}Nurlan et al. 1979). This decrease in protein synthesis is caused by a reduction in translational efficiency (Preedy et al. 1984b) possibly due to lower insulin and elevated glucagon levels. The effect of short-term fasting on protein degradation is controversial. Li and Wassner (1984) and Goodman et al. (1980) demonstrated that skeletal muscle proteolysis increases in rats fasted for 24 h. Emery et al. (1986) and Garlick et al. (1975) found that there was an abrupt rise in the rate of protein degradation of smooth muscle and liver of rats fasted for one day. However, Emery et al. (1986) detected no change while Millward et al. (1976) found a decrease in skeletal muscle proteolysis in rats fasted for two days. The reasons for these discrepancies are not clear but may arise from differences in experimental preparations. There is some evidence that reduced insulin levels in short-term fasting may accelerate proteolysis through alterations in the lysosomal pathway (Goldberg and Griffin 1977).

The decline in protein synthesis associated with short-term fasting is maintained during long term-fasting (El Haj et al. 1986; Golden et al. 1977). Urinary nitrogen excretion decreases in men fasted for 6 d (Saudek et al. 1976) and protein degradation decreases in chronically malnourished children (Golden et al. 1977). The decrease in protein degradation is likely associated with a reduction in the use of amino acids and an adaptation to fat as the principle energy source, thereby avoiding a life threatening loss of body protein. Some researchers have suggested that a reduction in thyroid activity may be responsible for the decreased rate of protein degradation in long-term fasting (Vignati et al. 1978; Goldberg et al. 1978).

Reducing the amount of protein in the diet while maintaining an adequate caloric intake results in a reduction in the rate of protein synthesis in the rat liver, kidney, heart, brain and skeletal muscles (Garlick et al. 1975). Rats on protein restricted diets exhibit a decline in skeletal muscle proteolysis and a rapid increase in liver protein breakdown (Garlick et al. 1975). The decline in muscle proteolysis helps to conserve muscle mass and protein content (Havenberg et al. 1975). Protein deficient diets are known to reduce thyroid activity which may decrease proteolysis through inhibition of lysosomal autophagy (Goldberg and Griffin 1977).

F. EFFECT OF ENVIRONMENTAL TEMPERATURE ON NITROGEN METABOLISM AND PROTEIN TURNOVER

1. Cold exposure

It has been well documented that both acute and chronic cold exposure induce an increase in urinary nitrogen excretion in the rat (Bavetta and Nimni 1964; Beaton 1963). Studies by Fauconneau and Arnal (1985) and Mathews and Haschemeyer (1978) have been carried out to examine the effect of cold exposure on protein synthesis in fish. Fauconneau and Arnal (1985) demonstrated that fish maintained in water at 10 C decreased liver, digestive tract and skeletal muscle protein synthesis by 66, 56, and 14 % respectively, when compared to fish maintained in water at 18 C. Fish in water at 5 C had a 96 % lower rate of liver protein synthesis than fish in water at 25 C (Mathews and Haschemeyer 1978). However, whether or not similar changes in protein synthesis occur in homeotherms exposed to reduced environmental temperatures remains unclear. Few researchers have examined the effect of cold exposure on protein turnover in mammals. Yousef and Luick (1969) found a 15 % increase in protein turnover in rats housed at 4 C when compared to rats housed at room temperature. However, their method of measuring protein turnover is no longer considered acceptable (Waterlow et al. 1978). Smith (1976) suggested that the negative nitrogen balance induced by cold exposure is due to an increase in peripheral tissue protein degradation, but did not try to measure this change directly with radioactive tracers. Thompson et al. (1987) demonstrated that myofibrillar protein degradation increased in calves and steers exposed to -10 C for 6 days and fed ad libitum or pair fed to animals at thermoneutrality by measuring a 61 % increase in urinary excretion of 3-methylhistidine. Millward et al. (1985) found a 19 and 20 % increase in skeletal muscle protein synthesis and degradation, respectively in rats exposed to 4 C for 14 d. Since the increase in degradation was greater than the increase in synthesis a net loss of muscle protein occurred. Millward et al. (1983) hypothesized that these changes in protein synthesis and protein degradation in the rat were due to an increased concentration of T_3 in the circulatory system. However, both of Millward's studies failed to demonstrate any statistically significant changes in protein synthesis or protein degradation during cold exposure of the rat. It is apparent that the relationship between protein turnover and cold exposure is still uncertain.

2. Heat exposure

Exposure of rats for 12 w (Hale and Mefferd 1963) or man for 24 h (Beisel et al. 1968) to high environmental temperature results in a negative nitrogen balance. During infection, the elevated body temperature associated with endotoxin causes a negative nitrogen balance in man (Beisel 1966). Garlick et al. (1980b) noted that infection causes an increase in both protein synthesis and degradation in man. It is uncertain whether long-term heat exposure would elicit a similar response since physiological adaptations act to maintain body temperatures at a normal level. Baracos et al. (1984) demonstrated that incubation of rat skeletal muscle in vitro at 39 C resulted in an increased rate of protein degradation (55 %) and decreased protein synthesis (33 %) when compared to muscles incubated at 33 C. Presently, it appears that no work has been done regarding the effect of heat stress on protein turnover in vivo.

F. OBJECTIVES OF THE PRESENT STUDY

From a review of the literature it was concluded that the single flooding dose (Garlick et al. 1980a) is the prefered method for the in vivo measurement of protein turnover in small animals. However, the extreme instability of the ninhydrin derivative used in this procedure is a major limitation. Therefore, the first objective of this study was to develop a high-performance liquid chromatographic method for the quantitative determination of phenylalanine.

From the literature it is apparent that several factors influence the rate of protein turnover. To date, researchers (Millward et al. 1985; Millward 1983; Yousef and Luick 1969) have failed to determine the relationship between cold exposure and protein turnover. There appears to be no studies which have examined the effect of heat exposure on protein turnover in vivo. The objectives of the present study also included:

(1) to determine if a plateau in the specific radioactivity of tissue free phenylalanine can be achieved after a flooding dose of ^{3}H-phenylalanine is injected intraperitoneally;

(2) to determine the effects of elevated and reduced environmental temperatures on protein turnover in several tissues of the laboratory rat.

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II. High Performance Liquid Chromatographic Analysis of ß-Phenylethylamine For the Estimation of In Vivo Rates of Protein Synthesis

A. INTRODUCTION

A number of techniques have been proposed for the measurement of protein synthesis in vivo. Garlick et al. (1980) developed a method whereby a single large dose of L-[^{3}H]-phenylalanine is intravenously pulse injected to flood all free amino acid pools in the body so that the specific radioactivity in each pool is similar to that in the plasma and so that the specific radioactivity decreases slowly in a linear manner. The relative simplicity of this method to provide an accurate value for the specific radioactivity of a precursor amino acid for protein synthesis has lead to its wide use, eg.(Brown et al. 1983; Preedy et al. 1986; Sampson et al. 1984).

Enzymatic conversion of the injected L-[^{3}H]-phenylalanine to L-[^{3}H]-tyrosine in the liver makes it necessary to isolate phenylalanine to obtain an accurate estimate of its specific radioactivity. Isolation is achieved by enzymatic decarboxylation of phenylalanine to ß-phenylethylamine followed by organic extraction. In the original method of Suzuki and Yagi (1976) developed a method to determine the amount of ß-phenylethylamine present in the organic extract which involved spectrofluorimetric analysis of a ninhydrin derivative of ß-phenylethylamine. This method of analysis is tedious and somewhat limited in output as the ninhydrin derivative is highly unstable in the presence of light and heat.

In this chapter an automated high-performance liquid chromatographic (HPLC) precolumn derivatization method routinely used in our laboratory for the measurement of ß-phenylethylamine is described. Approximately 100 samples can be analyzed per day.

B. MATERIALS AND METHODS

HPLC grade methanol, Brij 35 and tetrahydrofuran were obtained from Fisher Scientific (Edmonton, Alberta). All water was deionized with a Milli-Q water purification system. O-phthaldialdehyde, 2-mercaptoethanol, L-tyrosine decarboxylase, ethanolamine, and ß-phenylethylamine hydrochloride were purchased from Sigma (St. Louis, MO, U.S.A). L-[2,6-^{3}H] phenylalanine was obtained from Amersham (Amersham, U.K.) and its purity was checked following separation by HPLC using the conditions described. Fractions of column effluent were collected and counted. Radioactivity was only present in the phenylalanine fraction and no other peaks were detected.

Male Sprague-Dawley laboratory rats (Charles River, St. Constant, Quebec) were housed in individual cages and provided with water and a purified diet containing 20 % casein, ad libitum (Table II-1). Each rat was given an intraperitoneal injection of 150 umol phenylalanine per 100 g body weight, containing 50 uCi L-[2,6-^{3}H]-phenylalanine. Rats were killed 15 min after injection by cervical dislocation and the soleus, extensor digitorum longus and gastrocnemius muscles and the liver were immediately removed and placed in ice cold 2 % $HClO_4$ and stored at -70°C until analyzed.

Tissue samples were prepared in a manner similar to that reported by Garlick et al. (1980). Samples weighing between 40 and 400 mg were homogenized in 2 mL of 2 % ice cold $HClO_4$ with a glass to glass tissue homogenizer, while samples weighing more than 400 mg were homogenized in 3 mL 2 % $HClO_4$. The tissue samples were centrifuged at 2800 g for 15 min to percipitate the protein and the supernatant was decanted. Saturated potassium citrate was added to the supernatant in a quantity equivalent to one half of the supernatant volume resulting in the precipitation of $KClO_4$ at a pH of approximately 6. The sample was centrifuged at 2800 g for 15 min and the supernatant removed. Muscle supernatants were concentrated to approximately 1 mL in a water bath at 50 C under a stream of N_2. It was not necessary to concentrate the liver supernatants which were used to estimate free phenylalanine specific radioactivity.

The protein precipitate was washed three times by resuspending in 10 mL 2 % $HClO_4$ to ensure removal of free phenylalanine. The washed muscle and liver precipitates were hydrolyzed in 5 mL 6 N HCl for 24 h at 110 C. The HCl was subsequently removed by placing the samples in a vacuum oven overnight. The amino acids were resuspended in 1 mL 0.5 M sodium citrate at pH 6.3. The hydrolysate was used for estimating protein-bound phenylalanine specific radioactivity. Enzymatic conversion of phenylalanine to ß-phenylethylamine was performed by adding 0.5 mL L-tyrosine decarboxylase (2 unit mL^{-1})

suspended in 0.5 M sodium citrate, pH 6.3 containing 1 mg pyridoxal phosphate mL^{-1}, to both the supernatant and the hydrolysate. The samples were incubated overnight in a water bath at 50 C.

The extraction of ß-phenylethylamine was performed in a manner similar to that reported by Suzuki and Yagi (1976). ß-phenylethylamine was isolated by adding 0.5 mL 3N NaOH and 5 mL chloroform-n-heptane (1:3,v/v) to the incubated sample. The sample was shaken, centrifuged at 200 g for 5 min and the organic layer removed. The organic layer was shaken with 2.5 mL chloroform containing 2.0 mL 0.1N H_2SO_4. A 100 uL portion of the aqueous phase from tissue supernatants or a 1 mL portion from tissue protein hydrolysates, was placed in 10 mL Unisolve 1 and counted in a Beckmann LS5801 liquid scintillation spectrometer, while 100 uL aliquots were saved for analysis by HPLC.

Ethanolamine was used as an internal standard in all samples analyzed by HPLC. Stock solutions of ß-phenylethylamine (25 nmol mL^{-1}) in 0.1 N H_2SO_4 and ethanolamine (25 nmol mL^{-1}) in saturated potassium borate, pH 9.0 were prepared and used for calibration.

Samples containing 100 uL of tissue supernatant fluid and 50 uL of the internal standard were prepared and placed in 200 uL vials in an autoinjector at room temperature. Protein hydrolysate samples containing 50 uL of hydrolysate fluid, 1 mL of H_2O and 500 uL of the internal standard were placed in 2.0 mL vials in the autoinjector.

The HPLC system consisted of a Varian model 5000 liquid chromatograph coupled to a Fluoriochrom detector. The fluorimeter was set at an excitation range of 340-380 nm and an emission cutoff at 390 nm. All samples were injected by means of a Valco autoinjector equipped with a 25 uL loop. A C-18 reverse-phase Supelco column (75 x 4.6 mm I.D.; particle size, 3um) protected by a guard column (50 x 4.6 mm I.D.; particle size, 40-60 um) was used for all samples. Two solvents, solvent A, 0.1 M sodium acetate:methanol:tetrahydrofuran (900:95:5) (pH 7.2) and solvent B, methanol were used to form the following gradient; 40 % B at 0 min to 90 % B at 4 min, 90 % B from 4 min to 40 % B at 4.5 min and 40 % B from 4.5 to 10.3 min. The flow rate was set at 1.5 mL min^{-1}. A Hewlett-Packard 3350 series data system was used to record and integrate all peaks.

Derivatization of ß-phenylethylamine was performed using the o-phthaldialdehyde (OPA) reagent originally used for amino acid analysis by Jones et al. (1983). The fluoraldehyde reagent was prepared by dissolving 0.5 g o-phthaldialdehyde in 12.5 mL HPLC grade methanol, 0.5 mL 2-mercaptoethanol, 10 mL Brij 35 and 112 mL 0.04 M sodium borate (pH 9.5).

A Technicon (Tarrytown, NY, U.S.A.) autoanalyzer proportioning pump was used to draw the fluoraldehyde reagent and mix it via a T connector with the sample, just before injection on the column. A 15 sec delay time between the T connector and the column was adequate for derivatization. The autoanalyzer pump was controlled by the microprocessor on the chromatograph and was activated 30 sec before and left running for 30 sec after injection to clean the previous sample from the injection loop.

C. RESULTS AND DISCUSSION

The lengthy procedure required for the formation of the relatively unstable ninhydrin derivative used in the fluorimetric method of Suzuki et al. (1976) makes the analysis of ß-phenylethylamine inconvenient and time consuming. Using the present method, ß-phenylethylamine was found to be stable for at least 64 h in 0.1 N H_2SO_4 at room temperature permitting samples to be stored in an autosampler. Also, the time between derivatization and analysis was short and constant for each sample due to automation of the system, allowing reproducible quantitative analysis of the OPA-ß-phenylethylamine derivative.

Using the conditions described, the retention times of ethanolamine and ß-phenylethylamine were 2.80 min and 6.40 min, respectively. Figure II-1A illustrates the relative fluorescence response of the ethanolamine and the ß-phenylethylamine derivatives injected on the HPLC column. Figures II-1B, II-1C and II-1D illustrate that ß-phenylethylamine was successfully isolated from muscle and liver cell fluid and muscle hydrolysates respectively, after incubation with tyrosine decarboxylase. Chromatograms of liver and muscle hydrolysate samples which were extracted but not incubated with tyrosine decarboxylase showed no trace of material with a retention time similar to that of ß-phenylethylamine (Figure II-2).

The present method offers several advantages over the original ninhydrin method. The fluorescent ninhydrin derivative is highly unstable in the presence of light and the analysis time must be kept constant by use of a stop watch. In the present method the autosampler kept analysis time constant and the addition of an internal standard could be used as a correction factor for the instability of the OPA derivative. In the ninhydrin method of Suzuki and Yagi (1976) it was necessary to incubate the sample for a period of 1 h in order to form a fluorescent derivative. In the present method this step requires a negligible amount of time since derivatization occurs within 15 sec. With the ninhydrin method the operator was unable to monitor the efficiency of the extraction procedure. With the present method, the inclusion of a strip chart recorder made it possible to monitor the adequacy of extraction of ß-phenylethylamine enabling one to determine the presence of contaminating tyramine that would contribute radioactivity to the aliquot counted resulting in an apparent over-estimation of phenylalanine specific radioactivity. The chromatograms in Figure II-1 illustrate that not all of the compounds containing a primary amine group were removed by the extraction procedure. Standards containing tyrosine, tyramine and phenylalanine were analyzed and their retention times did not correspond to any of these compounds. Since tyrosine, phenylalanine or tyramine are not present in these fractions they do not introduce additional radioactivity that could interfere with the calculation of phenylalanine specific radioactivity. However, it was noted that occasional improper extraction would result in the contamination of the extract with tyrosine, phenylalanine and tyramine which contribute to radioactivity in the sample counted.

Since ß-phenylethylamine is a neurotransmitter normally present in tissues and body fluids, endogenous ß-phenylethylamine may interfere with the calculation of phenylalanine specific radioactivity. Preedy et al. (1985) estimated that endogenous ß-phenylethylamine in brain tissue accounted for only 0.1 % of the phenylalanine present after decarboxylation. Tsuji et al. (1986) have recently shown that free ß-phenylethylamine is present in the brain and liver at concentrations averaging 4.61 and 14.70 ng g wet wt^{-1}, respectively. Figure II-3A demonstrates that considerable endogenous free ß-phenylethylamine is present in the liver. It was found that adequate amounts were present to meaningfully interfere with the calculation of the amount of phenylalanine in samples of tissue fluids, consequently resulting in an underestimation of the specific radioactivity of free phenylalanine leading in an over estimation of the fractional rate of protein synthesis. The sensitivity of the present method permits this error to be readily corrected by measuring the amount of endogenous ß-phenylethylamine present in the tissue supernatants prior to incubation with tyrosine decarboxylase. Figure II-3B illustrates that only trace amounts of free endogenous ß-phenylethylamine are present in the muscle supernatants. Tsuji et al. (1986) were unable to detect ß-phenylethylamine in muscle but did detect ß-phenylethylamine in blood. It is probable that the increased sensitivity of our method accounts for the detection of the trace amounts of free endogenous ß-phenylethylamine in the extracellular fluid. The amount of endogenous ß-phenylethylamine in the muscle supernatant is too small to interfere with the estimation of the specific radioactivity of free phenylalanine.

Serial dilution of ß-phenylethylamine standards resulted in a linear relative fluorescence response from 8 to 840 pmol injection^{-1} (Figure II-4). The coefficient of variation for 25 pmol ß-phenylethylamine was 2.1 % (n=6). The original fluorimetric assay (Suzuki and Yagi, 1976) had a detection limit of 100 pmol. The present system is sensitive enough to detect as little as 0.5 pmol ß-phenylethylamine. Although not utilized in this study, the extreme sensitivity of the present system would make it ideal for the quantitative measurement of endogenous ß-phenylethylamine in biological tissues. The percent recovery for the organic extraction has been previously determined by Suzuki and Yagi (1976).

A limiting feature of using the ninhydrin-spectrofluormetric method in the estimation of protein synthesis is that a relatively large proportion of the sample is required to determine the amount of ß-phenylethylamine present, leaving a relatively small proportion to determine the radioactivity. In the present method the amount of radioactivity available for counting was increased in two ways. In the ninhydrin method it is necessary to use 1 mL (of supernatant) or 200 uL (of hydrolysate) for the quantitative determination of phenylalanine. The increased senstivity of the present method permits the use of only 100 uL and 50 uL of supernatant and hydrolysate, respectively, leaving a greater proportion of the sample for the determination of radioactivity. Concentration of the supernatant fluid to approximately 1 mL along with halving the solvent volumes in the organic extraction increased the concentration of ß-phenylethylamine and hence the radioactivity in both the supernatant and hydrolysate fluid samples approximately ten fold. With 200 mg of muscle tissue 16,000-20,000 c.p.m. and 8,000-12,000 c.p.m. were present in the supernatant and hydrolysate, respectively. This is an improvement over the original method where 200 mg of tissue contained 1,000-2,000 c.p.m and 100-200 c.p.m. in the supernatant and hydrolysate, respectively.

Laboratories using the ninhydrin method are capable of measuring the amount of ß-phenylethylamine in 30-40 samples each day. With the present method and the addition of an autosampler to the HPLC system it is possible to determine the amount of ß-phenyethylamine in 90-100 samples per day.

Following the establishment of the present technique in our laboratory, Tsuji et al. (1986) published an HPLC method for the analysis of ß-phenylethylamine which required an analysis time of 16 min with a retention time of 13.5 min for ß-phenylethylamine. Approximately 30 samples per day could be analyzed by this method. The isolation of free endogenous ß-phenylethylamine by this method is relatively more labour intensive than by the simple organic extraction used in the present method as their samples are passed through columns containing Dowex 50 and Amberlite CG-50 with evaporation to dryness at each step. It is of importance to note that chromatograms of the material isolated by the organic solvent procedure are much cleaner than chromatograms of the material isolated by the column chromatography method of Tsuji et al. (1986). The detection limit for the method of Tuji et al. (1986) was 400-500 pg sample^{-1} compared to no more than 100 pg sample^{-1} in the present method.

The high resolving capability and sensitivity of the present method, coupled with full automation and reduced analysis time, offer substantial advantages over previously published methods for the isolation and determination of ß-phenylethylamine in biological samples. The method is ideally suited for studies of in vivo protein turnover in which the specific radioactivity of free and peptide-boound phenylalanine must be accurately determined in large numbers of samples.

Table II-1

AIN-76™ Purified Diet

Ingredient	%
Casein	20.0
DL-Methionine	0.3
Cornstarch	15.0
Sucrose	50.0
Fiber	5.0
Corn oil	5.0
AIN Mineral mix*	3.5
AIN Vitamin mix*	1.0
Choline bitartrate	0.2
	100.0

* AIN mineral and vitamim mix were obtained from ICN biomedical chemicals (Cleveland, Ohio, U.S.A.).

Figure II-1. Chromatograms obtained from standards and biological samples incubated with tyrosine decarboxylase. (A) Standard containing ß-phenylethylamine (PEA). (B) Muscle cell fluid from extensor digitorum longus. (C) Liver cell fluid. (D) Muscle hydrolysate from extensor digitorum longus. All samples were chromatographed on a 3 um Supelco C-18 column (75 x 4.6 mm I.D.) at a flow rate of 1.5 mL min^{-1}. The internal standard ethanolamine (EA) was added to all samples and standards.

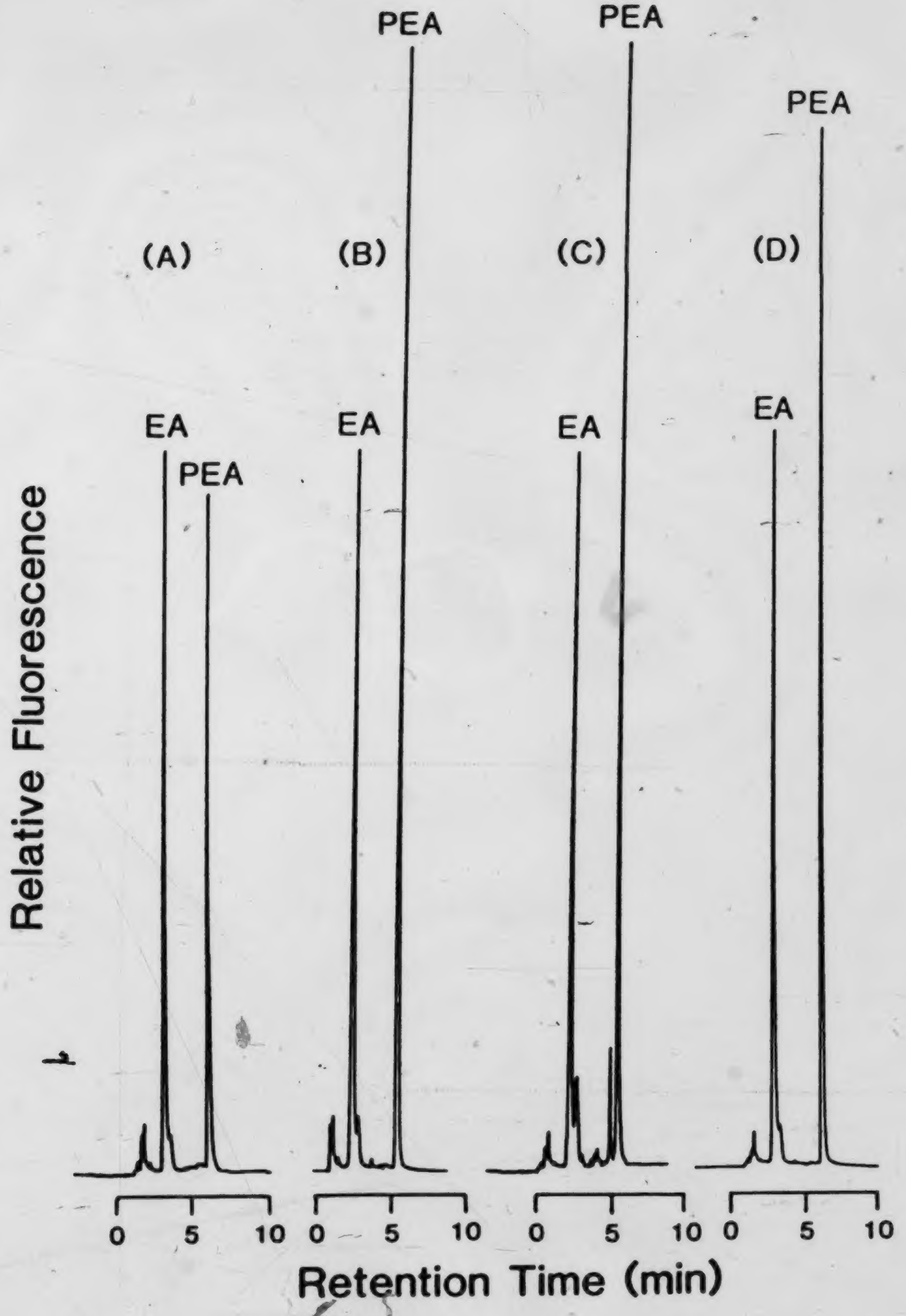

PEA
PEA
PEA
(A)
(B)
(C)
(D)
EA
PEA
EA
EA
EA
Relative Fluorescence
0
5
10
0
5
10
0
5
10
0
5
10
Retention Time (min)

Figure II-2. Chromatogram of hydrolysate samples which were extracted but not incubated with tyrosine decarboxylase illustrating the absence of ß-phenylethylamine. (A) Liver. (B) Skeletal muscle.

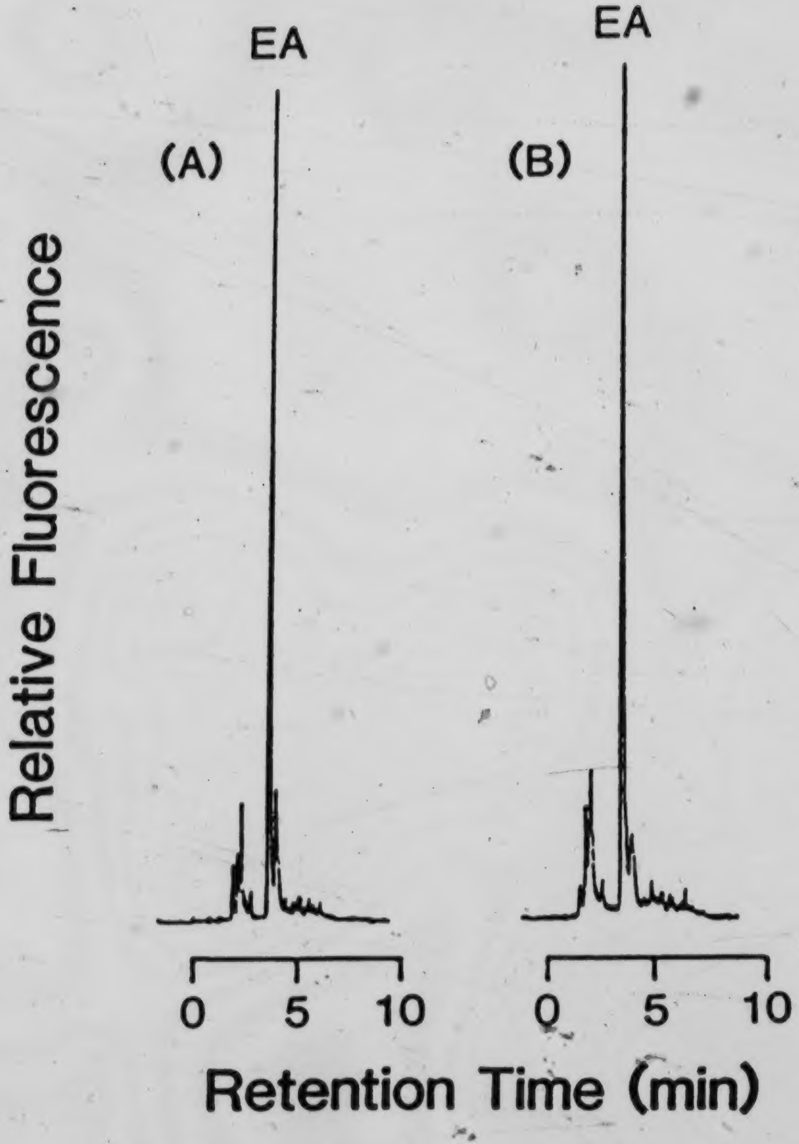

EA
EA
(A)
(B)
Relative Fluorescence
0
5
10
0
5
10
Retention Time (min)

Figure II-3. Chromatograms of supernatant fluid not incubated with tyrosine decarboxylase. (A) Liver. (B) Skeletal muscle.

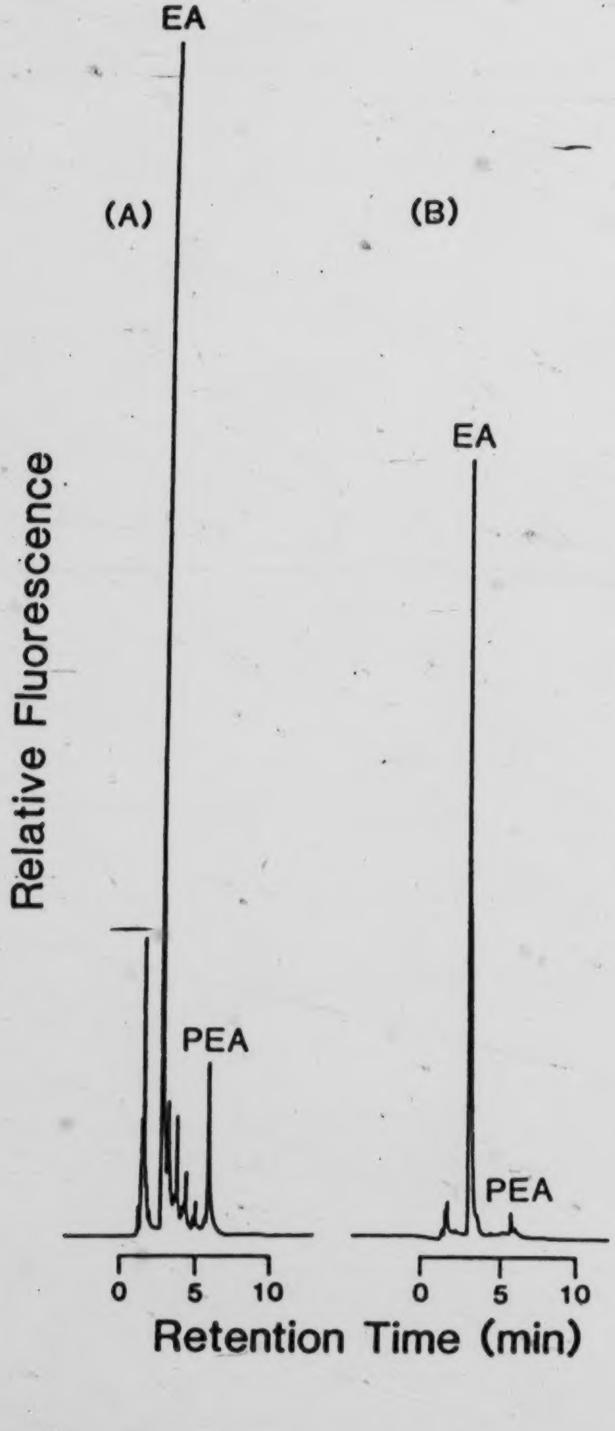
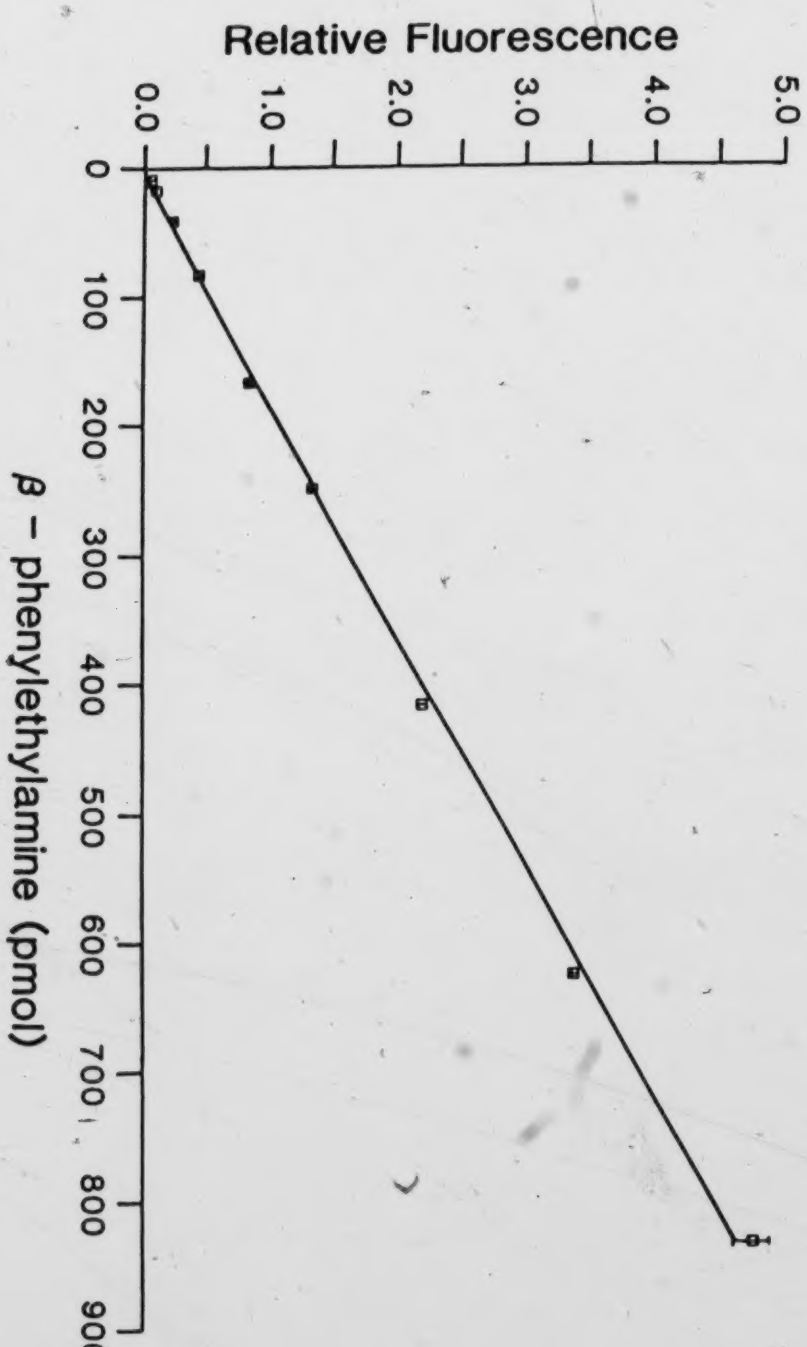

(A)
EA
PEA
(B)
EA
PEA
Relative Fluorescence
0
5
10
0
5
10
Retention Time (min)

Figure II-4. Relationship between the concentration of ß-phenylethylamine and relative fluorescence. Serial dilutions of ß-phenylethylamine where analyzed by HPLC. Means of triplicates are plotted, bars indicate standard error. Method as described in text.


Relative Fluorescence
0.0
1.0
2.0
3.0
4.0
5.0
0
100
200
300
400
500
600
700
800
900
β - phenylethylamine (pmol)

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III. The Effect of Environmental Temperature on Protein Turnover in Several Body Tissues of the Laboratory Rat.

A. INTRODUCTION

Animals subjected to stressors such as hot or cold environmental temperature exhibit a number of physiological and biochemical responses. For example, food intake increases dramatically when rats are exposed to cold temperatures (Klain et al. 1963; Yousef and Luick 1969) and decreases when rats are exposed to hot temperatures (Hale and Mefferd 1958; Sugahara et al. 1969). Cold exposure elevates the metabolic rate of rats (Depocas 1956) and cattle (Bell et al. 1975) while heat exposure causes a reduction in the metabolic rate of mice (Hennycuick 1967) and hamsters (Cassuto 1968). Hormonal parameters are altered by exposure of animals to increased and decreased environmental temperatures. For example, thyroid activity in man (Eastman et al. 1974), rats (Straw 1969), and cattle (Christopherson et al. 1979) is enhanced during cold exposure and repressed in cattle (Magdub et al. 1982), pigs (Evans and Ingram 1977) and sheep (Sutherland and Irvine 1974) during heat exposure. The effects of these and other physiological and biochemical changes on protein metabolism have only been partially characterized.

Most researchers examining animal protein metabolism in cold and hot environments have concentrated on the changes in plasma and tissue amino acid concentration and nitrogen metabolism. Early studies by William et al. (1950) and Rangneker and Dugal (1958) demonstrated that cold exposure of rats caused a decrease in plasma and tissue amino acid concentrations. More recently, Bell et al. (1975) did not observe any difference in the release of amino acids from the hind limb of steers exposed to 0 C for 4 d. Klain et al. (1963) noted that the activity of liver enzymes associated with amino acid metabolism are increased in rats during chronic cold exposure. This finding is supported by the observation that urinary nitrogen excretion is increased in rats exposed to cold for 7 and 21 d (Beaton, 1963; Bavetta and Nimni 1964). Most of these observations are consistent with an increased rate of skeletal muscle protein breakdown during exposure to reduced environmental temperatures.

Exposure to elevated environmental temperatures also causes an increase in urinary nitrogen excretion. Holmes (1973) found that pigs housed at 34 C for 3 d had a 10 % greater urinary nitrogen excretion than pigs housed at 25 C. Similar increases in urinary nitrogen excretion have been found in rats exposed to 35 C for 24 w , (Hale and Mefferd 1963)) and humans exposed to 39.4 C for 6 h (Beisel et al. 1968). In general, these studies suggest that protein catabolism is accelerated by both heat exposure.

Few researchers have used radioactively labeled precursor amino acids to measure the rate of protein turnover in animals exposed to heat and cold temperatures. Initially, Chaffee et al. (1968), Chaffee et al. (1969) and Yousef and Luick (1969) used ^{75}Se-selenomethionine to measure protein turnover, a procedure which is no longer considered valid (Waterlow et al. 1978). These researchers concluded that protein turnover was increased in rats exposed to cold for 10 w and decreased in rats chronically exposed to heat. Using more acceptable methods of measuring protein turnover, Haschemeyer et al. (1979) found that acute heat exposure reduces the rates of protein synthesis in fish gill, skeletal muscle and liver. Further work by Mathews and Haschemeyer (1978) and Fauconneau and Arnal (1985) demonstrated that acute cold exposure inhibits protein synthesis in fish. However, it is not known whether exposure to hot and cold environmental temperatures leads to similar changes in protein turnover in homeotherms.

Millward et al. (1985) found that there was a nonsignificant increase in muscle protein synthesis and degradation in rats housed at 4 C for 14 d. Other researchers have demonstrated that acute cold exposure of humans (Carli and Itibia 1986) and chronic cold exposure of calves (Thompson et al. 1987) causes an increase in myofibrillar protein breakdown. Baracos et al. (1984) have shown that increasing the temperature of the incubation media in vitro, decreases protein synthesis and increases protein degradation in rat skeletal muscle.

Researchers have been unable to clearly demonstrate the relationship between environmental temperature and protein turnover in vivo. Furthermore, researchers have not attempted to measure the alterations in protein turnover which may occur during long term adaptation to high or low environmental temperatures. In the present study an attempt was made to determine the fractional and absolute rates of protein synthesis and degradation in different skeletal muscles, heart and liver of rats exposed to hot or cold temperatures for a 25 d period.

B. MATERIALS AND METHODS

The sources of laboratory rats, L-[2,6-^{3}H]phenylalanine and other chemicals have been described in Chapter II.

One hundred and forty-four male Sprague-Dawley rats, averaging 45 ± 10 g body weight were obtained at 21 d of age. All rats were provided with water and a synthetic casein based ration ad libitum during the adaptation period as described in Appendix 1.

After adaptation, 36 rats were transferred to a 10 C cold room and a second group of 36 rats were placed in a 30 C hot room. After one day, the temperature of the cold room was lowered to 5 C, while that of the hot room was raised to 35 C. A control group of 36 rats remained in a room at 25 C. Rats in all three groups were provided with water and a diet ad libitum throughout the experiment. The feed intake of all three groups was recorded daily at 1100 h. A fourth group of 36 rats was maintained at 25 C, provided with water ad libitum and was fed an amount equivalent to that consumed by rats in the hot environment the previous day. The weight gain of rats in all four groups was recorded every second day at 1400 h.

Six rats from each group were killed by cervical disslocation on days 0, 5, 10, 15, 20 and 25 of the trial. Rats killed on days 5, 10, 15 and 20 of the experiment were given an intraperitoneal injection of L-[2,6-^{3}H]phenylalanine 15 min before sacrifice as described in

Chapter II. All rats were retained in their specified treatment rooms during injection. The soleus, extensor digitorum longus, gastrocnemius and heart muscles and the liver were immediately removed, weighed and placed in ice cold 2 N $HClO_4$ and stored in a freezer at -70 C. The heart was rinsed in ice-cold saline and blotted on a paper towel to ensure that all blood was removed prior to weighing. Whole blood was obtained from each rat by severing the subclavian artery and vein and collecting the blood in evacuated tubes coated with heparin. The tubes were mixed and immediately centrifuged at 3500 g for 15 min at 5 C. The plasma was removed and stored at -70 C until analysis.

Total plasma concentration of L-triiodothyronine (T_3) was determined using a Coat-A-Count^{TM} radioimmunoassay kit obtained from Diagnostic Products Corporation (Los Angeles, CA., U.S.A). Protein content of the muscles and liver was estimated by the method of Lowry et al. (1951).

Tissue samples from rats which received an intraperitoneal injection of ^{3}H-phenylalanine were prepared and the specific radioactivities of the free and protein-bound phenylalanine were determined as reported in Chapter II. Fractional rates of protein synthesis were calculated as described in Appendix 1. In addition, absolute rates of protein synthesis were obtained by multiplying the fractional synthesis rate by the total amount of protein contained within each tissue (Waterlow et al. 1978). Absolute rates of protein synthesis were expressed as mg protein synthesized day^{-1}.

Daily growth rates (K_g) in each tissue were determined by regression analysis and expressed as a percentage of the protein mass accumulated during the experimental period. The fractional degradation rate (K_d) was estimated indirectly by subtracting the fractional growth rate (K_g) from the fractional synthesis rate (K_s) (Turner and Garlick 1974; Millward et al. 1975; Kelly and Goldspink 1982).

All results were expressed as means ± S.E.M. and were analyzed by one way analysis of variance and SNK multiple comparison of means as described by Steele and Torrie (1980).

C. RESULTS

Tables which report the mean values ± S.E.M for each of the figures in this Chapter are presented in Appendix 2.

Daily Feed Intake

The daily feed intake of rats in the three different temperatures was recorded from day 2 to 24 (Figure III-1). The feed intake of rats exposed to 5 C was higher ($P<0.05$) than that of control rats and rats exposed to 35 C on each day during the trial. Rats exposed to 35 C had a lower ($P<0.05$) feed intake than that of control rats between days 4 and 24.

Mean Body Weight

The mean body weights of rats in cold, control, hot and pair fed groups are illustrated in Figure III-2. There was no difference ($P>0.05$) in the mean body weight of rats on day 5. Between days 10 and 25 the mean body weights of rats exposed to 35 C and pair fed rats were lower ($P<0.05$) ~~than those of~~ control rats. The mean body weights of rats exposed to 5 C were lower ($P<0.05$) than those of control rats between days 15 and 25. Rats in the cold group had higher ($P<0.05$) mean body weights than rats in the hot between days 20 and 25 and pair fed rats between days 15 and 25.

Rate of Gain

Control rats tended to have a higher rate of gain than rats in the other three groups (Figure III-3). The rate of gain of rats exposed to 5 C was lower ($P<0.05$) than that of control rats on all but days 10, 16, 18, 20, and 24 of the trial, while the rate of gain of rats exposed to 35 C was lower ($P<0.05$) than that of control rats on all but days 2 and 18. Pair fed rats had a significantly lower rate of gain ($P<0.05$) than control rats between days 4 and 22. On day 2, the rate of gain of rats in the hot and pair fed groups was higher ($P<0.05$) than that of rats in the cold group. The rate of gain of rats exposed to 5 C was higher ($P<0.05$) on days 4 to 10 and on days 14 and 16. Rats exposed to cold temperature had a higher ($P<0.05$) rate of gain than pair fed rats between days 6 and 20, while rats exposed to 35 C had a higher ($P<0.05$) rate of gain than pair fed rats on days

6, 8, 12, 16 and 20.

Tissue Weights

The mean weights of tissues from rats in the cold, control, hot and pair fed groups are shown in Figure III-4. Compared to those from control rats, the extensor digitorum longus muscles from rats exposed to 5 C were lighter ($P<0.05$) on days 5, 15 and 25 while those from rats exposed to 35 C were lighter ($P<0.05$) on day 25. These muscles were also lighter ($P<0.05$) in pair fed rats than in control rats on days 15 and 25. On day 5, the extensor digitorum longus muscles of both heat-exposed and pair fed rats were heavier ($P<0.05$) than those of rats exposed to 5 C.

The soleus muscles from cold-exposed, heat-exposed and pair fed rats were lighter ($P<0.05$) than those of control rats on days 10, 15 and 25. On days 5 and 15 the soleus muscles from rats exposed to 35 C were heavier ($P<0.05$) than those from rats exposed to 5 C. On day 15, rats which were pair fed possesed soleus muscles which weighed significantly more ($P<0.05$) than those from rats exposed to 5 C .

The gastrocnemius muscles from rats exposed to 5 C were lighter ($P<0.05$) than those from control rats on all days with the exception of day 10. These muscles were lighter in both heat-exposed and pair fed rats ($P<0.05$) in comparison to control rats between days 15 and 25. On day 5, the gastrocnemius muscles from heat-exposed and pair fed rats were larger ($P<0.05$) than those from rats exposed to 5 C.

The hearts from rats exposed to 5 C were heavier ($P<0.05$) than those from control, heat-exposed and pair fed rats throughout the trial. The hearts from both heat-exposed and pair fed rats were lighter ($P<0.05$) than those from control rats between days 10 and 25.

There was no difference ($P>0.05$) in liver weight between rats exposed to 5 C and control rats. Rats which were pair fed or exposed to 35 C possesed livers which were lighter ($P<0.05$) than those of control rats on each day of the trial with the exception of rats exposed to 35 C on day 5. The livers from rats exposed to 35 C were larger ($P<0.05$) than those from pair fed rats on days 5, 10 and 15.

When adjusted for body weight, the weights of the extensor digitorum longus, soleus and gastrocnemius muscles from rats pair fed or exposed to 35 C were all larger than those from control rats and rats exposed to 5 C between days 10 and 25 (Figure III-5). The increased weight of hearts from rats exposed to 5 C was even more prevalent when tissue weight was adjusted for body weight. For example, hearts from rats exposed to cold temperatures were 23 % larger ($P<0.05$) than those from control rats on day 25. When adjusted for body weight, the livers from rats exposed to 5 C weighed more than those from control rats throughout the trial. Adjustment for body weight also emphasized the reduced size of livers from rats which were exposed to hot temperature or pair fed. For example, on day 20 the decrease in the weight of livers from heat-exposed and pair fed rats relative to control rats was 25 ($P<0.05$) and 37 % ($P<0.05$), respectively.

Fractional Rate of Protein Synthesis

The fractional rates of protein synthesis in the rat tissues are shown in Table III-1. The fractional rate of protein synthesis in the extensor digitorum longus muscle was not significantly altered by exposure to cold or hot temperatures or by pair feeding. However, there was a trend for the fractional rate of protein synthesis in this muscle from cold-exposed, heat-exposed and pair fed rats to be lower than that of control rats between days 5 and 15. For example, on day 15 the fractional rate of protein synthesis in the extensor digitorum longus muscles from rats exposed to 5 C, 35 C or pair fed was 21, 16 and 27 % lower respectively, than that of controls.

On day 5, the fractional rate of protein synthesis in the soleus muscles from pair fed rats was greater ($P<0.05$) than that from rats exposed to 5 and 35 C. On average, the fractional rates of protein synthesis in the soleus muscles from rats exposed to 35 C or pair fed tended to be lower than those from control rats.

Exposure of rats to cold or hot environmental temperatures or pair feeding did not produce any changes ($P>0.05$) in the fractional rate of protein synthesis in the gastrocnemius muscle. However, there was a trend for the fractional rate of protein synthesis in this muscle in heat-exposed and pair fed rats to be lower than that of controls.

In general, the fractional rate of protein synthesis in the hearts from both control and cold-exposed rats was greater than those from heat-exposed and pair fed rats ($P<0.05$). The fractional rate of protein synthesis in the hearts from both cold-exposed and control rats was 39 ($P<0.05$) and 34 % ($P<0.05$) higher respectively, than those from pair fed rats on day 10. On day 15, decreases in the fractional rate of protein synthesis relative to control rats in the hearts from rats exposed to 35 C or pair fed were 37 ($P<0.05$) and 33 % ($P<0.05$), respectively. On day 20, the fractional rate of protein synthesis in the hearts from rats exposed to 5 C was 30 ($P<0.05$) and 49 % ($P<0.05$) higher than those from both control and pair fed rats, respectively.

The fractional rate of protein synthesis in the liver was not altered ($P>0.05$) by exposing rats to cold or hot temperatures or by pair feeding. On average, there was a trend for the fractional rate of protein synthesis in the livers from rats exposed to 5 or 35 C to be higher than those from control rats. For example, on day 15 the fractional rate of protein synthesis in the livers from both cold and heat-exposed rats was 28 and 27 % higher respectively than those from control rats .

Absolute Rate of Protein Synthesis

On average, the absolute rate of protein synthesis in the extensor digitorum longus muscles was decreased ($P<0.05$) in rats exposed to 5 and 35 C in comparison to control rats (Figure III-6). For example, on day 15 the absolute rate of protein synthesis of the extensor digitorum longus muscles from rats exposed to 5 or 35 C was decreased relative to those from control rats by 29 and 42 %, respectively.

On average, the absolute rate of protein synthesis in the soleus muscles from rats exposed to 5 C, 35 C or pair fed was lower ($P<0.05$) in comparison to those from control rats (Figure III-7). For example, on day 15 the absolute rate of protein synthesis in the soleus muscles from rats exposed to 5 or 35 C was 29 and 28% lower respectively, than those from control rats.

Absolute rates of protein synthesis in the gastrocnemius muscle of cold-exposed, heat-exposed and pair fed rats were significantly lower ($P<0.05$) on average than those of control rats. The absolute rate of protein synthesis in the gastrocnemius muscles from rats exposed to 35 C or pair fed was decreased on day 20 by 47 ($P<0.05$) and 49 % ($P<0.05$), respectively in comparison to those from control rats (Figure III-8). On day 10, the absolute rate of protein synthesis in the gastrocnemius muscles from rats exposed to 5 C tended to be higher than those from rats exposed to 35 C or pair fed.

Exposure of rats to 5 C caused a dramatic increase in the absolute rate of protein synthesis in the heart (Figure III-9). For example on day 20, the absolute rate of protein synthesis in the hearts from rats exposed to 5 C was 46 % greater ($P<0.05$) than those from control rats. Overall, the hearts from rats which were exposed to 35 C or pair fed had a lower ($P<0.05$) absolute rate of protein synthesis than those from control rats. For example, after 15 d these rates in the hearts from rats which were pair fed or exposed to 35 C decreased relative to those from control rats by 48 ($P<0.05$) and 41 %, respectively.

Overall, the absolute rate of protein synthesis in the livers from rats exposed to 5 C was higher ($P<0.05$) than the rates from rats exposed to 35 C or pair fed. (Figure III-10). The overall absolute rate of protein synthesis in the livers from control rats did not differ ($P>0.05$) from those of rats exposed to 5 C, 35 C or pair fed. The absolute rate of protein synthesis in the livers from rats exposed to 5 C was greater ($P<0.05$) than those from pair fed rats on day 20.

Fractional Rates of Protein Degradation

The fractional rates of protein degradation in rat tissues are reported in Table III-2. Exposure of rats to 5 or 35 C for 20 d tended to cause a gradual increase in the fractional rate of protein degradation in the extensor digitorum longus muscle Throughout the experimental period. On day 20, in rats exposed to 5 or 35 C the fractional rate of protein degradation in this muscle was increased relative to that of control rats by 16 and 18 %, respectively, while in pair fed rats it remained similar to control rats throughout the trial.

The fractional rates of protein degradation in the soleus muscles from rats exposed to 5 C tended to be greater than those from control rats on days 10 and 20. When compared to control rats, the fractional rate of protein degradation in the soleus muscles from rats exposed to 35 C tended to show a gradual decrease throughout the trial, while that of pair fed rats tended to show a gradual decrease until day 15 and increased on day 20.

The fractional rate of protein degradation in the gastrocnemius muscles from rats exposed to 5 C was quite similar to those from control rats throughout the trial. At 20 d, the fractional rate of protein degradation in the gastrocnemius muscles from rats exposed to 35 C or pair fed tended to decrease relative to those from control rats.

Exposure of rats to 5 C for 20 d tended to increase the fractional rate of protein degradation in the heart in comparison to that of control rats, while rats exposed to 35 C did not differ relative to control rats in this respect. The fractional rate of protein degradation in the hearts of pair fed rats tended to be greater than those from control rats on day 5.

The fractional rate of protein degradation in the livers from rats exposed to 5 C tended to be higher than in those from control rats throughout the experiment, while exposure of rats to 35 C tended to result in a decrease between days 10 and 20. There was little difference between the fractional rate of protein degradation in the livers from pair fed and control rats.

Absolute Rate of Protein Degradation

There was a trend for the absolute rate of protein degradation in the extensor digitorum longus muscles from rats exposed to 5 or 35 C to be less than those from control rats between days 5 and 15 (Figure III-11). On day 20, there was a tendency for the fractional rate of protein degradation in the extensor digitorum longus muscles from rats exposed to 35 C to be higher than those from control rats. Absolute rates of protein degradation tended to be greater in this muscle from pair fed rats on days 5 and 10 in comparison to control rats.

Exposure of rats to 5 C tended to decrease the absolute rate of protein degradation in the soleus muscles in comparison to those from control rats on days 5 and 15 (Figure III-12). Exposure of rats to 35 C tended to result in a decrease in this respect relative to control rats on all days with the exception of day 20. There was a trend for pair fed rats to have a lower absolute rate of protein degradaton in the soleus muscles relative to those from control rats on days 15 and 20.

Exposure of rats to cold or hot temperatures or pair feeding tended to decrease the absolute rate of protein degradation in the gastrocnemius muscles in comparison to those from control rats (Figure III-13). The absolute rate of protein degradation in the gastrocnemius muscles from rats exposed to 5 C tended to be greater than those from rats exposed to 35 C or pair fed on days 10, 15 and 20.

Exposure of rats to 5 or 35 C tended to cause a large increase and decrease, respectively in the absolute rate of protein degradation of the heart relative to control values (Figure III-14). For example, after 20 days the absolute rate of protein degradation in the hearts from rats exposed to 5 or 35 C was 70 % higher and 41 % lower, respectively, than those from control rats. The absolute rate of protein degradation in the hearts of pair fed rats tended to be lower than those from control rats throughout the trial.

The absolute rate of protein degradation in the livers from rats exposed to cold temperature tended to be much greater than those from control rats between days 10 and 20 (Figure III-15). The absolute rate of protein degradation in the livers from rats exposed to 35 C or pair fed tended to be reduced in comparison to those from control rats.

Tri-iodothyronine Concentrations

Exposure of rats to 5 C caused an increase in plasma tri-iodothyronine (T_3) concentration throughout the trial (Figure III-16). The maximum increase in plasma T_3 concentration occurring on day 5 of cold exposure represented a mean rise in plasma T_3 concentration of 37.5 % ($P<0.05$) above the mean plasma T_3 concentration of control rats. On day 25 the plasma T_3 concentration of rats exposed to 35 C or pair fed decreased ($P<0.05$) in comparison to that of control rats by 45 and 44 %, respectively.

D. DISCUSSION

Exposure of rats to extreme cold or hot temperatures leads to a retardation of growth and development (Hale et al. 1959; Ray et al. 1968; Roubicek et al. 1964). In the present study the mean body weight (Figure IV-2) and the rate of gain (Figure IV-3) of rats which were exposed to 5 or 35 C was reduced in comparison to control rats. The need to utilize more energy for maintenance of body temperature rather than for growth would explain why rats exposed to 5 C, despite their increased feed intake, had a lower growth rate than control rats. The similarity in body weight and rate of gain between rats exposed to 35 C and pair fed rats suggests that the reduced growth in rats at 35 C is related to a lower feed intake. A reduction in feed intake would lower the amount of heat produced by digestion, thereby enabling the rats at 35 C to more readily regulate their body temperature (Chaffee et al. 1969). In addition, it has been demonstrated that rats exposed to high temperature have a lower maintenance requirement than those housed at room temperature (Alhassan et al. 1976).

Clearly, skeletal muscle, heart muscle and liver respond differently upon exposure to cold or hot temperatures (Figure IV-4). On average, the weights of the three skeletal muscles studied were reduced ($P<0.05$) in rats exposed to 5 or 35 C in comparison to those from control rats. The extensor digitorum longus and soleus are predominantly a white fiber-type muscle and red fiber-type muscle respectively, while the gastrocnemius muscle is a mixture of the two fiber types. The present results indicate that the weights of muscles of all fiber types are similarily affected by cold or heat exposure. Heroux (1958) also found that the size of the soleus muscle was reduced by 30 % in rats exposed to 6 C in comparison to rats at 30 C, while the whole body muscular mass was reduced by 25 %.

Compositional analysis indicated that protein deposition in skeletal muscle was reduced by cold exposure (Heroux 1958). The present study supports this finding and indicates that exposure of rats to 35 C causes a similar reduction in skeletal muscle weight.

When adjusted for body weight, the weights of the three skeletal muscles tended to be higher in rats exposed to 35 C or pair fed relative to those from rats exposed to 5 C or control rats (Figure IV-5). Although not measured directly, rats exposed to 5 C and control rats appeared to have more body fat than rats exposed to 35 C and pair fed rats. The increased body weight in rats exposed to 5 C and control rats resulting from greater fat deposition would account for the reduction in body weight adjusted skeletal muscle weight as compared to rats exposed to 35 C and pair fed.

In the present study, the fractional rates of protein synthesis and protein degradation reported for control rats in Table III-1 and Table III-2, respectively correspond to values obtained by researchers using an intravenous flooding dose of H^3-phenylalanine. For example, previous researchers have found that the fractional rates of protein synthesis and degradation in the soleus and gastrocnemius muscles were 6.1, 5.3 (El Haj et al. 1986) and 10.9, 6.31 % day^{-1} (Odedra et al. 1983), respectively. Preedy et al. (1984) estimated that the fractional rate of protein synthesis and protein degradation in the hearts from young rats was 8.95 and 6.45 % day^{-1}, respectively. M^cNurlan and Garlick (1980) determined that the fractional rate of protein synthesis in rat liver was 105 % day^{-1}.

To the best of the author's knowledge no researchers have used the intravenous single flooding dose technique for the estimation of the rate of protein syntheisis and degradation in the extensor digitorum longus muscle or the rate of protein degradation in the liver.

A reduction in the fractional (Table III-1) and absolute (Figure III-6) rates of protein synthesis in the extensor digitorum longus muscles from cold-exposed, heat-exposed and pair fed rats in comparison to those from control rats may have contributed to the observed differences in muscle weight. Over the 20 d trial the average absolute rate of protein synthesis in the extensor digitorum longus muscle from rats exposed to 5 or 35 C was lower ($P<0.05$) than in muscles from control rats.

The average fractional rate of protein synthesis in the soleus muscle was quite variable on individual days throughout the trial. However, the overall absolute rates of protein synthesis during the 20 d trial were lower in rats exposed to 5 C ($P<0.05$), 35 C ($P<0.05$) and pair fed ($P<0.05$) than in control rats (Figure III-7).

The fractional rate of protein synthesis in the gastrocnemius muscles tended to decrease in rats exposed to 35 C in comparison to those from control rats as determined in the preliminary study (see Appendix 1). The fractional rate of protein synthesis in the gastrocnemius muscles from pair fed rats also tended to be less than those from control rats (Table III-1). Over the 20 d trial the average absolute rate of protein synthesis in the gastrocnemius muscles was decreased in rats exposed to 5 C ($P<0.05$), 35 C ($P<0.05$)

or pair fed ($P<0.05$) in comparison to those from control rats (Figure III-8). The observation that both the fractional and absolute rates of protein synthesis were reduced in both rats exposed to 35 C and pair fed suggests that the decline in protein synthesis in the gastrocnemius muscles from rats exposed to 35 C can partially be attributed to reduced feed intake. Other researchers (Millward et al. 1976; Garlick et al. 1975) have demonstrated that a decline in energy intake can cause a reduction in the fractional rate of protein synthesis in the gastrocnemius muscles from rats.

The fractional (Table III-2) and absolute rates of protein degradation in the three skeletal muscles tended to be quite variable during the experiment. During the 20 d trial exposure of rats to 35 C tended to cause a reduction in the average fractional rate of protein degradation in the extensor digitorum longus and soleus muscles when compared to control values (Figure III-11, III-12). Absolute rates of protein degradation in the gastrocnemius muscles from rats exposed to 5 C, 35 C and pair fed rats tended to be lower than those from control rats on each day during the trial (Figure III-13). Millward et al. (1983) found that cold exposure caused a nonsignificant increase in the fractional rate of protein synthesis and degradation in the gastrocnemius muscles from rats exposed to 4 C for 14 days. Millward et al. (1985) hypothesized that these changes in protein synthesis and degradation were a result of an increased plasma concentration of T_3 during cold exposure. In the present study, the plasma concentration of T_3 was increased by cold exposure (Figure III-16) However, our results suggest that protein synthesis and degradation in skeletal muscle may be inhibited by both cold and heat exposure.

The plasma concentration of T_3 was lower ($P<0.05$) in rats exposed to 35 C (Figure III-16) which confirms previous studies by Magdub et al. (1982) and Hurley et al. (1980). It has been shown that a decline in the plasma concentration of T_3 is associated with a decrease in skeletal muscle protein synthesis and degradation (Brown et al. 1983). It is possible that the decrease in the plasma level of T_3 is partially responsible for the overall decline in skeletal muscle protein synthesis and degradation during heat exposure.

In the present study, exposure of rats to 5 C caused a dramatic increase ($P<0.05$) in the size of the heart. Heroux and Gridgeman (1958) and Hale et al. (1959) found similar increases in the weight of the heart from rats exposed to cold temperature. Hearts from rats exposed to high environmental temperature were smaller than those of control rats (Figure III-4) confirming the study of Ray et al. 1976. However, when corrected for body weight there was little difference between the weight of hearts in rats exposed to 35 C and control rats.

The overall average fractional (Table III-1, III-2) and absolute rates (Figure III-9, III-14) of protein synthesis and degradation in the heart during the 20 d trial were increased by cold exposure ($P<0.05$). Previous studies have demonstrated that increased contractile work can increase the rate of skeletal muscle protein synthesis in vitro (Smith et al. 1983; Palmer et al. 1981 and in vivo (Laurent et al. 1978). In addition, it has been shown that increased contractile work can enhance the rate of protein degradation in the skeletal muscle of fowl (Laurent et al. 1978). Researchers have demonstrated that in rats (Depocas et al. 1957) and cattle (Bell et al. 1975) cold exposure increases the metabolic rate and thus the amount of contractile work performed by the heart. It is possible that the increased contractile work in a cold environment stimulates the rate of protein synthesis and degradation in the heart.

This hypothesis is supported by the observation that on average the fractional and absolute rates of protein synthesis ($P<0.05$) and degradation in the hearts from rats exposed to 35 C were lower than those from control rats. Heat exposure is known to cause a decrease in the metabolic rate and the amount of contractile work performed by the heart (Cassuto 1968; Cassuto and Chaffee 1966). With a decline in the amount of contractile work performed by the heart, one would expect a decrease in the rate of protein synthesis and degradation.

The fractional and absolute rates of protein degradation in the hearts from pair fed rats were on average lower than those from control rats. This reduction suggests that the decline in protein synthesis and degradation in the hearts of rats exposed to 35 C could partly be due to a reduction in food intake. Other researchers (Garlick et al. 1975; Preedy et al. 1984) have found that a reduction in the feed intake of rats decreases the rate of protein synthesis in the heart.

When adjusted for body weight, the weight of the livers in rats exposed to 5 C were greater than those from control rats. Beaton

(1963) and Hale et al. (1959) found similar increases in the weight of the liver in rats exposed to cold environmental temperature. In agreement with past studies by Ray et al. (1968) and Hale et al. (1959), the present results demonstrate that heat exposure decreases the weight of liver adjusted for body weight in rats (Figure III-5).

The overall average fractional rate of protein synthesis tended to increase in the liver during the 20 d trial when rats were exposed to 5 or 35 C in comparison to livers from control rats (Table III-1). This finding confirms the results which were obtained in the preliminary study (see Appendix 1). On the other hand, in comparison to control rats the absolute rate of protein synthesis in the liver tended to increase in rats exposed to cold temperature and decrease in rats exposed to hot temperature (Figure III-10). It is not necessary for absolute and fractional rates of protein synthesis to correspond since absolute rates of protein synthesis are influenced by changes in tissue weight. In the present study, the difference between protein synthesis and degradation was greater in rats exposed to 5 C than in those exposed to 35 C. Therefore, more protein was deposited in the livers of rats exposed to 5 C resulting in a greater liver weight.

In general, the changes in protein breakdown in liver parallel the changes in synthesis. Fractional rates of protein breakdown in the livers from rats exposed to 5 or 35 C tended to be greater in comparison to the livers from control rats (Table III-2). When protein degradation was expressed on an absolute basis, it tended to be higher in rats exposed to 5 C but lower in rats exposed to 35 C as compared to that from control rats (Figure III-15). This reflects the larger and smaller liver weights which were present in rats exposed to cold and hot temperatures, respectively. Whitten et al. (1970) attributed the changes in hepatic amino acid catabolism during cold and heat exposure to changes in caloric intake. These authors demonstrated that the activity of several hepatic enzymes such as transaminases are increased in rats exposed to cold temperature due to an increase in feed intake. This finding suggests that the metabolic activity of the liver is enhanced during cold exposure. This greater metabolic demand resulting from a higher caloric intake may explain why the rates of protein synthesis and degradation in the liver are increased by cold exposure.

In the present study, the similarity in the values for the absolute rates of protein synthesis and degradation in rats exposed to 35 C and pair fed rats supports the suggestion that alterations in protein turnover during heat exposure are partially related to caloric intake. The reduced feed intake of rats in the hot environment would reduce the metabolic demand and likely result in less protein being deposited in the liver. This reduced rate of protein deposition is reflected in the lower absolute rates of protein synthesis and degradation in the liver. However, fractional rates of protein synthesis and degradation indicate that protein turnover is increased in the livers from rats exposed to hot temperature. This stimulation of protein turnover may originate from internal metabolic activites rather than those related to diet. Whitten et al. (1970) found that the activity of urea cycle enzymes were enhanced in rats exposed to 35 C. This finding suggests that as in cold exposure, the metabolic activity of the liver may also be increased by heat exposure, possibly leading to an increased fractional rate of protein synthesis and degradation. In addition, 25-40 % of the protein synthesized in the liver is exported (Odedra et al. 1983). Therefore, it is possible that the fractional rate of export protein synthesis increases without an increase in the synthesis of fixed liver proteins. If this occurred, the fractional rate of protein synthesis in the liver could increase without resulting in an increase in the weight of the liver.

Millward et al. (1983) hypothesized that an elevated plasma T_3 concentration was responsible for increasing the rate of protein synthesis and degradation in skeletal muscles from rats exposed to cold temperatures. However, an increase in plasma T_3 concentration is only one of many changes in hormone concentration resulting from cold exposure. Therefore, the changes in protein turnover in skeletal muscle during cold exposure may be regulated by other hormones. The present study suggests that protein synthesis and degradation may decrease in the skeletal muscles from rats exposed to cold or hot temperatures. Insulin has been shown to increase the rate of protein synthesis in skeletal muscle (Stirewalt and Low 1983; Airhart et al. 1982). Smith et al. (1982) found that plasma insulin concentration was reduced by cold exposure. A decrease in plasma insulin concentration may reduce the rate of protein synthesis in skeletal muscle during cold exposure. In addition, failure to find a change in the urinary excretion of 3-methylhistidine, an index of myofibrilar protein breakdown, suggests that skeletal muscle protein breakdown is not accelerated by cold exposure in rats (Smith et al. 1982). Several studies have demonstated an increase in urinary nitrogen excretion (Beaton 1963; Bavetta and Nimni 1964) during cold exposure. According to the evidence presented here, an acceleration in urinary nitrogen excretion during cold exposure may result from a greater feed intake and an accelerated rate of protein turnover in the liver and heart.

In conclusion, it appears that cold exposure may decrease the absolute rate of protein synthesis in skeletal muscle, while increasing it in the heart and liver. Cold exposure tended to decrease the absolute rate of protein degradation in skeletal muscle and increase it in heart and liver. On average, heat exposure decreased the absolute rate of protein synthesis and degradation in muscle, heart and liver. Similar decreases in the absolute rate of protein synthesis and degradation in pair fed rats suggested that part of the decreases noted in rats exposed to 35 C were a result of reduced caloric or amino acid intake.

Figure III-1. Feed intake (g day $^{-1}$) of rats housed at three different temperatures; cold (5 C), control (25 C) and hot (35 C). Mean are values from 6 to 36 rats as reported in the Materials and Methods section. Feed intake of rats housed in the cold and in the hot environments were significantly higher ($P<0.05$) and significantly lower ($P<0.05$) respectively, than in rats housed in the control environment.

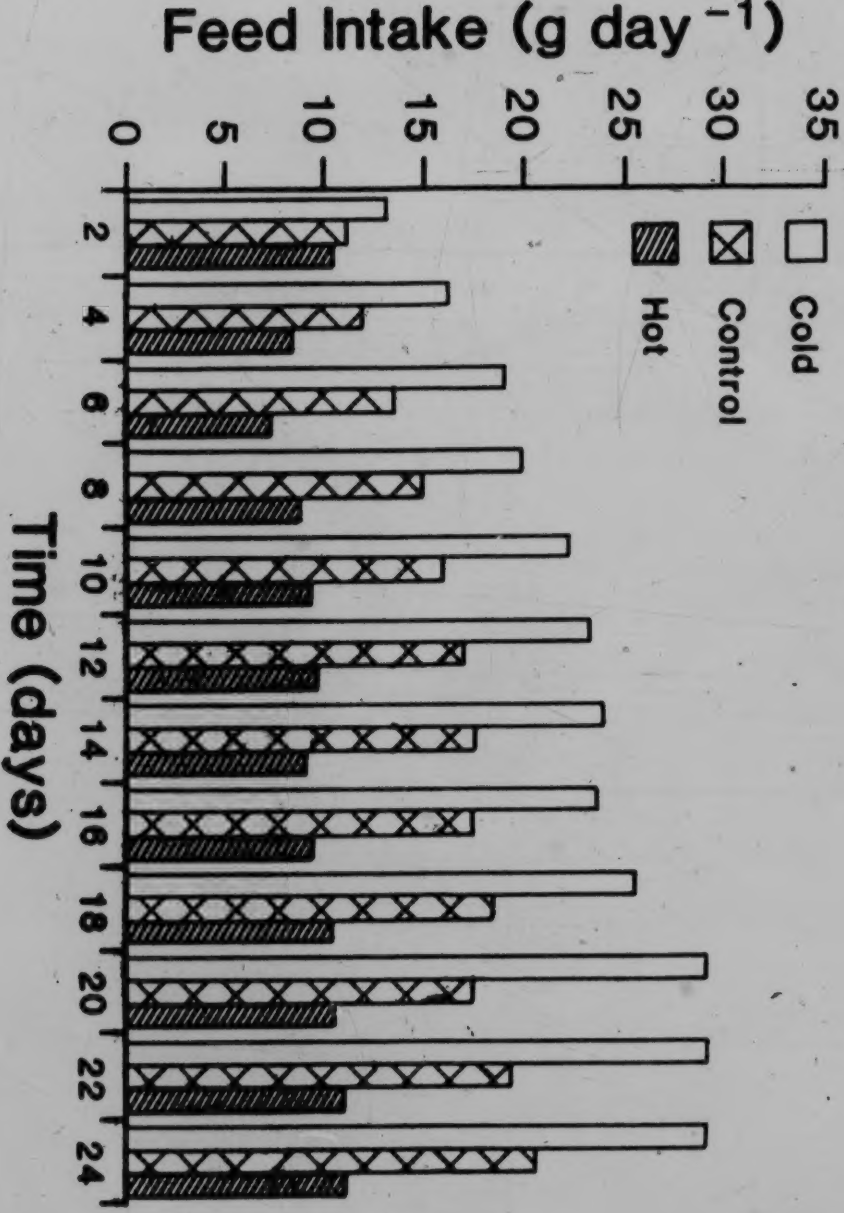

Feed Intake (g day $^{-1}$)
0
5
10
15
20
25
30
35
Cold
Control
Hot
2
4
6
8
10
12
14
16
18
20
22
24
Time (days)

Figure III-2. Mean body weight (g) of rats housed in cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 6 to 36 rats as reported in the Materials and Methods section.

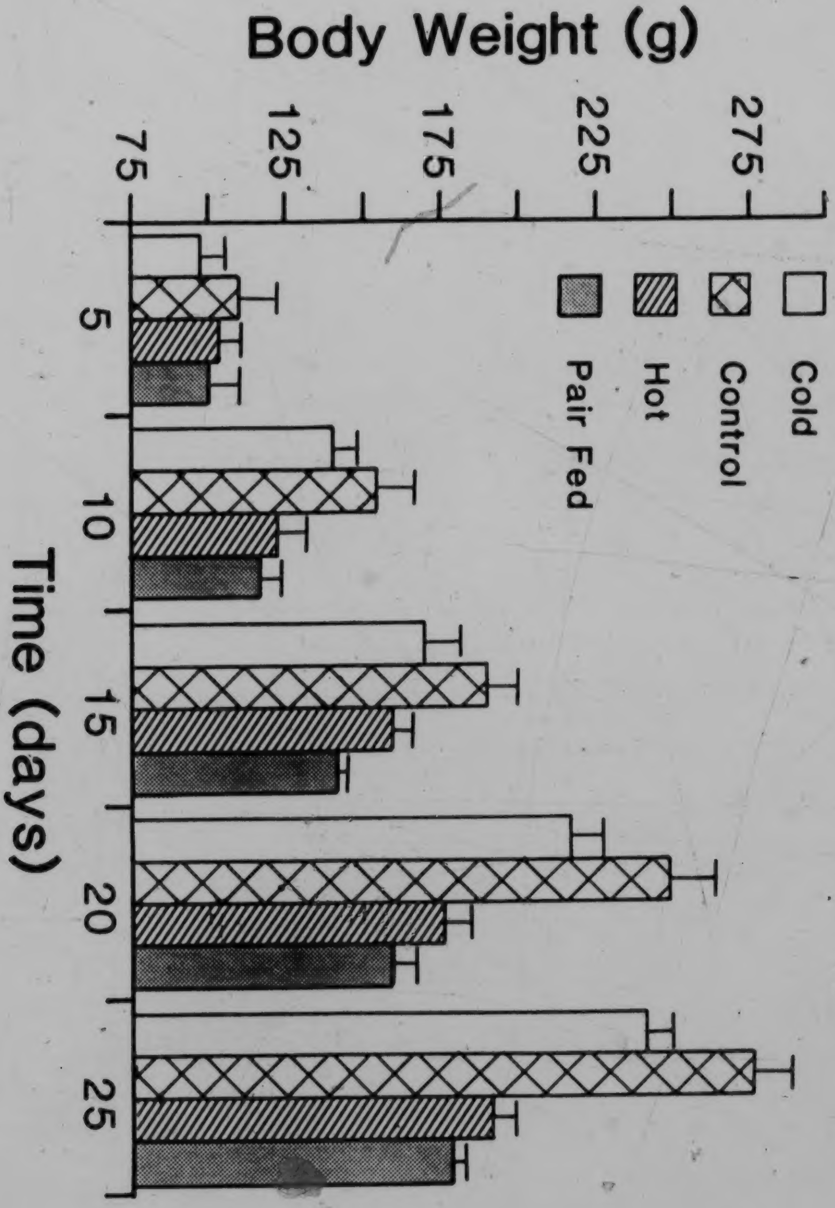

Body Weight (g)
75
125
175
225
275
Time (days)
5
10
15
20
25
Cold
Control
Hot
Pair Fed

Figure III-3. Daily rate of body weight gain (g day $^{-1}$) in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 6 to 36 rats as reported in the Materials and Methods section.

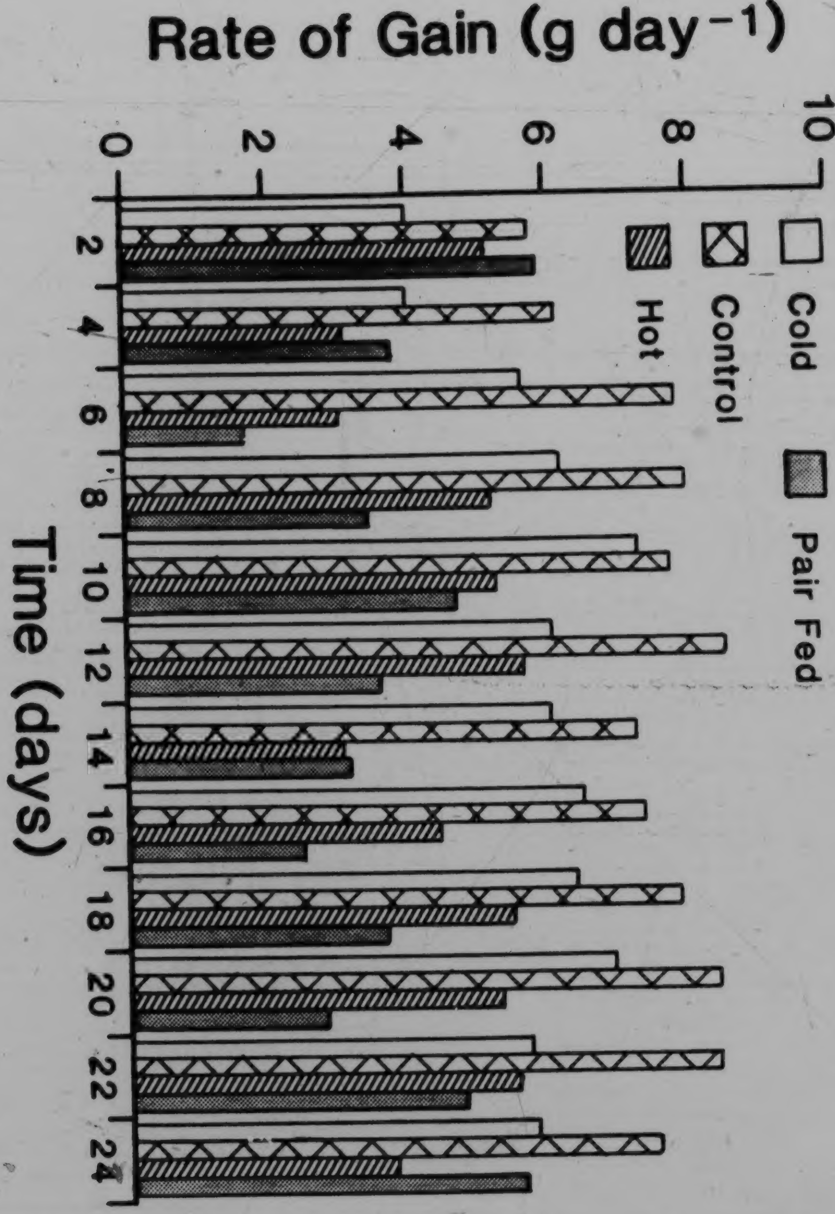

Rate of Gain (g day^{-1})
0
2
4
6
8
10
☐ Cold
☒ Control
▨ Hot
▩ Pair Fed
2
4
6
8
10
12
14
16
18
20
22
24
Time (days)

Figure III-4. Weight of tissues (mg) in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 5 to 6 rats. Bars represent standard error of the means (SEM).

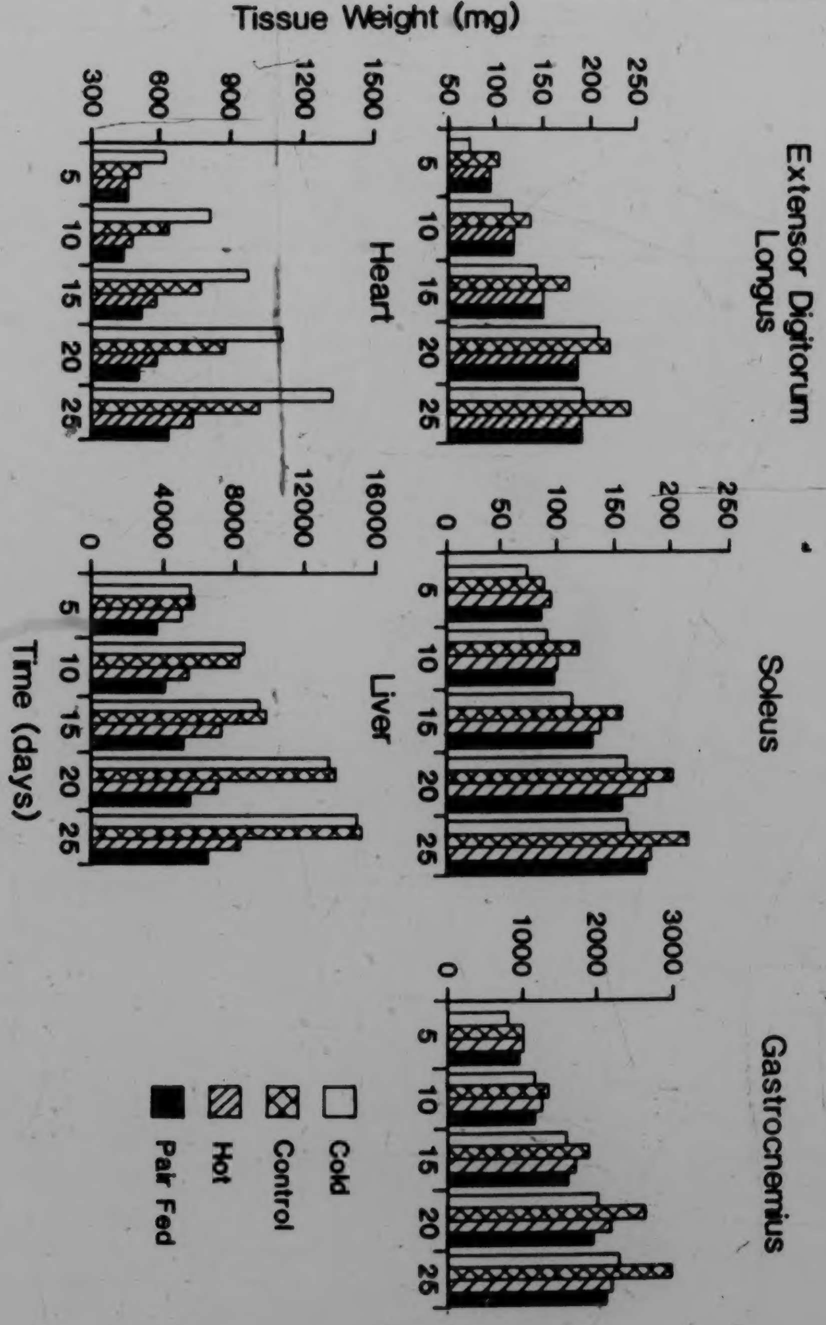

Tissue Weight (mg)
Extensor Digitorum Longus
50
100
150
200
250
Soleus
0
50
100
150
200
250
Gastrocnemius
0
1000
2000
3000
Heart
300
600
900
1200
1500
Liver
0
4000
8000
12000
16000
5
10
15
20
25
Time (days)
Cold
Control
Hot
Pair Fed

Figure III-5. Weight of tissues (mg $\cdot$ 100 g body weight^{-1}) adjusted for body weight in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 5 to 6 rats. Bars represent Standard error of the mean (SEM).

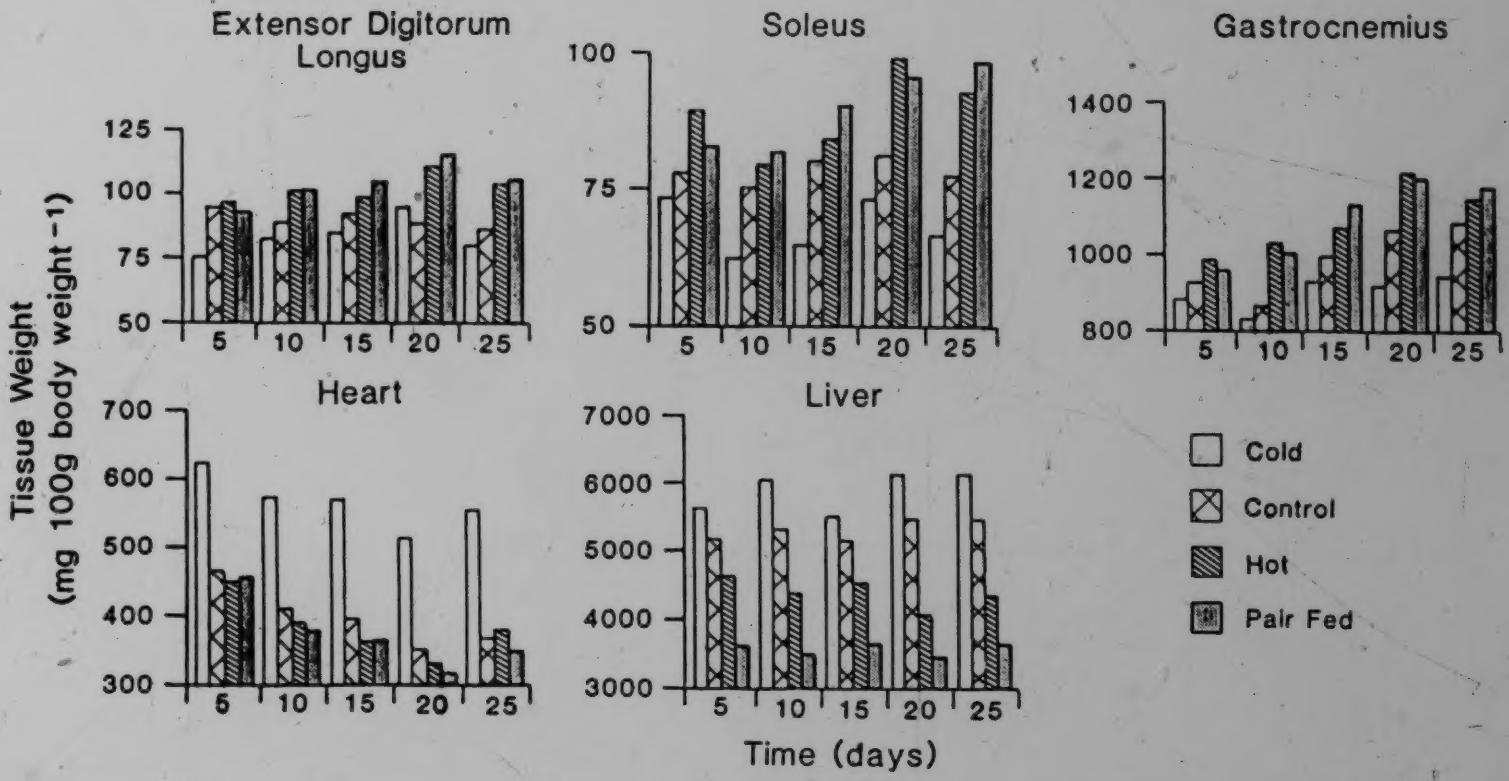

Extensor Digitorum Longus
Soleus
Gastrocnemius
Heart
Liver
Tissue Weight (mg 100g body weight -1)
125
100
75
50
100
75
50
1400
1200
1000
800
700
600
500
400
300
7000
6000
5000
4000
3000
5
10
15
20
25
Time (days)
Cold
Control
Hot
Pair Fed

Table III-1. Fractional rate of protein synthesis (% day^{-1}) in tissues from rats.[a]

Tissue		Time (days)			
		5	10	15	20
Extensor Digitorum	Cold	5.9	6.9	6.3	6.2
Longus		(.65)	(.54)	(.32)	(.57)
	Control	9.0	7.7	8.0	6.0
		(1.1)	(.65)	(.90)	(.55)
	Hot	8.6	6.9	6.7	6.2
		(1.1)	(.65)	(.71)	(1.1)
	Pair Fed	7.6	5.8	5.8	5.3
		(.68)	(.32)	(.51)	(.89)
Soleus	Cold	8.9 A	10.1	9.6	8.6
		(.76)	(.70)	(.79)	(.65)
	Control	12.7 AB	9.9	10.0	6.6
		(1.0)	(.97)	(.46)	(.90)
	Hot	8.9 A	8.6	8.6	7.3
		(1.2)	(1.1)	(.76)	(.84)
	Pair Fed	12.2 B	9.1	7.4	9.1
		(.97)	(.73)	(1.3)	(1.7)
Gastrocnemius	Cold	9.0	13.3	11.1	12.1
		(1.5)	(1.3)	(1.6)	(1.8)
	Control	11.5	12.4	12.2	11.7
		(1.7)	(1.8)	(.65)	(2.1)
	Hot	9.5	11.1	10.1	8.5
		(1.5)	(1.5)	(1.8)	(1.0)
	Pair Fed	9.5	11.2	10.7	8.7
		(.62)	(1.5)	(1.2)	(1.5)
Heart	Cold	11.8	10.1 A	9.0 AB	11.4 A
		(1.4)	(.82)	(1.4)	(1.2)
	Control	10.7	9.4 A	10.3 A	8.0 B
		(.82)	(.72)	(1.2)	(.85)
	Hot	8.7	7.9 AB	6.9 B	7.9 AB
		(.95)	(.86)	(.38)	(1.5)
	Pair Fed	10.0	6.2 B	6.5 B	5.8 B
		(1.8)	(.28)	(.36)	(.52)
Liver	Cold	178.1	193.0	205.[illegible]	168.1
		(50.2)	(68.6)	(40.3)	(22.2)
	Control	131.2	181.0	148.0	142.3
		(42.1)	(24.4)	(15.3)	(31.9)
	Hot	119.6	203.8	189.9	192.2
		(26.7)	(64.7)	(30.7)	(55.4)
	Pair Fed	148.7	212.9	180.5	126.1
		(13.8)	(57.3)	(25.1)	(23.6)

[a] mean values from 3-6 rats

( ) standard error of the mean

A,B - values with different superscripts are significantly different from each other at $P<0.05$.

Figure III-6. Absolute rate of protein synthesis (mg day^{-1}) in the extensor digitorum longus muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 3 to 6 rats. Overall, the absolute rate of protein synthesis in the extensor digitorum longus muscles of rats exposed to 5 C and 35 C were significantly lower ($P<0.05$) in comparison to control rats. Bars represent SEM.

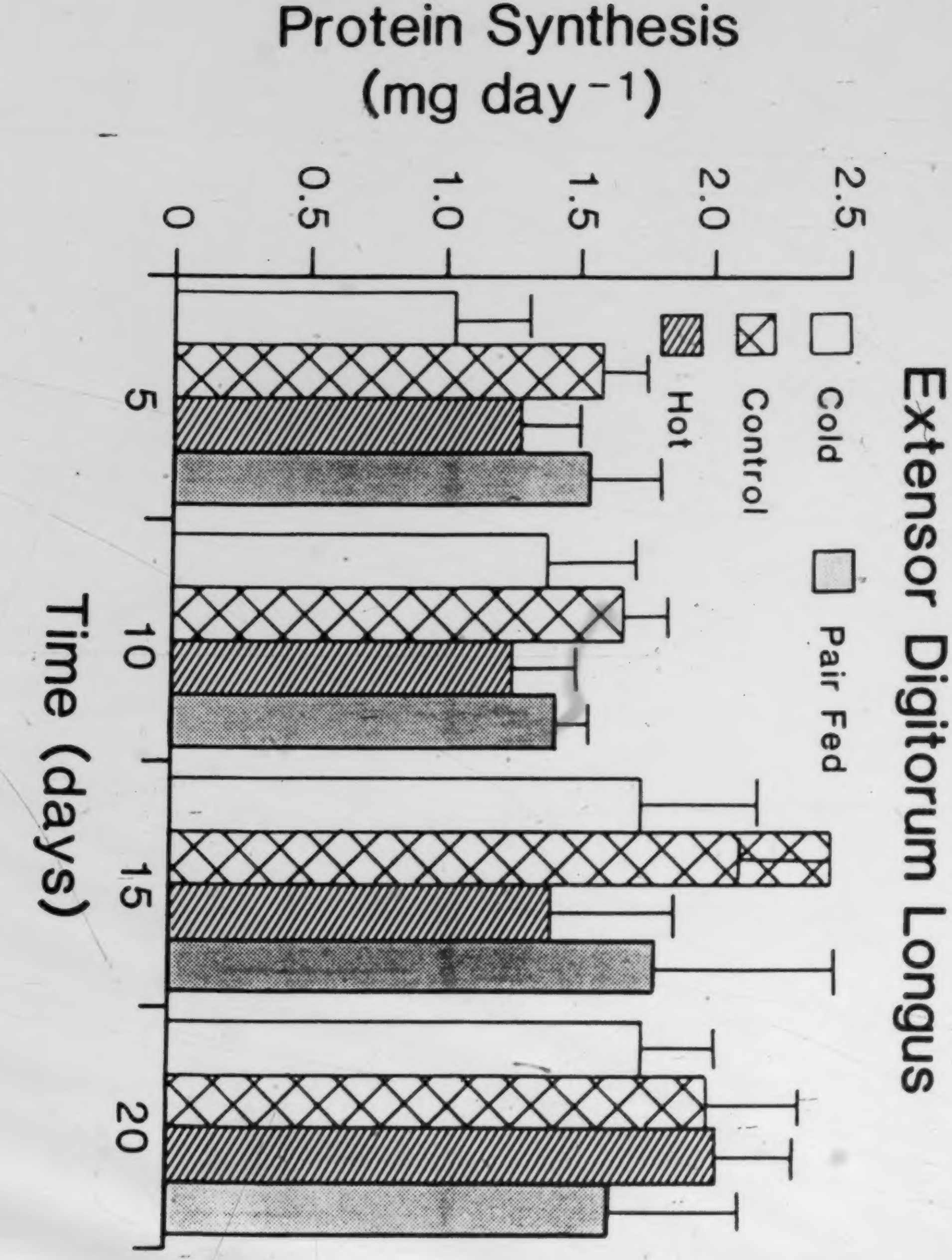
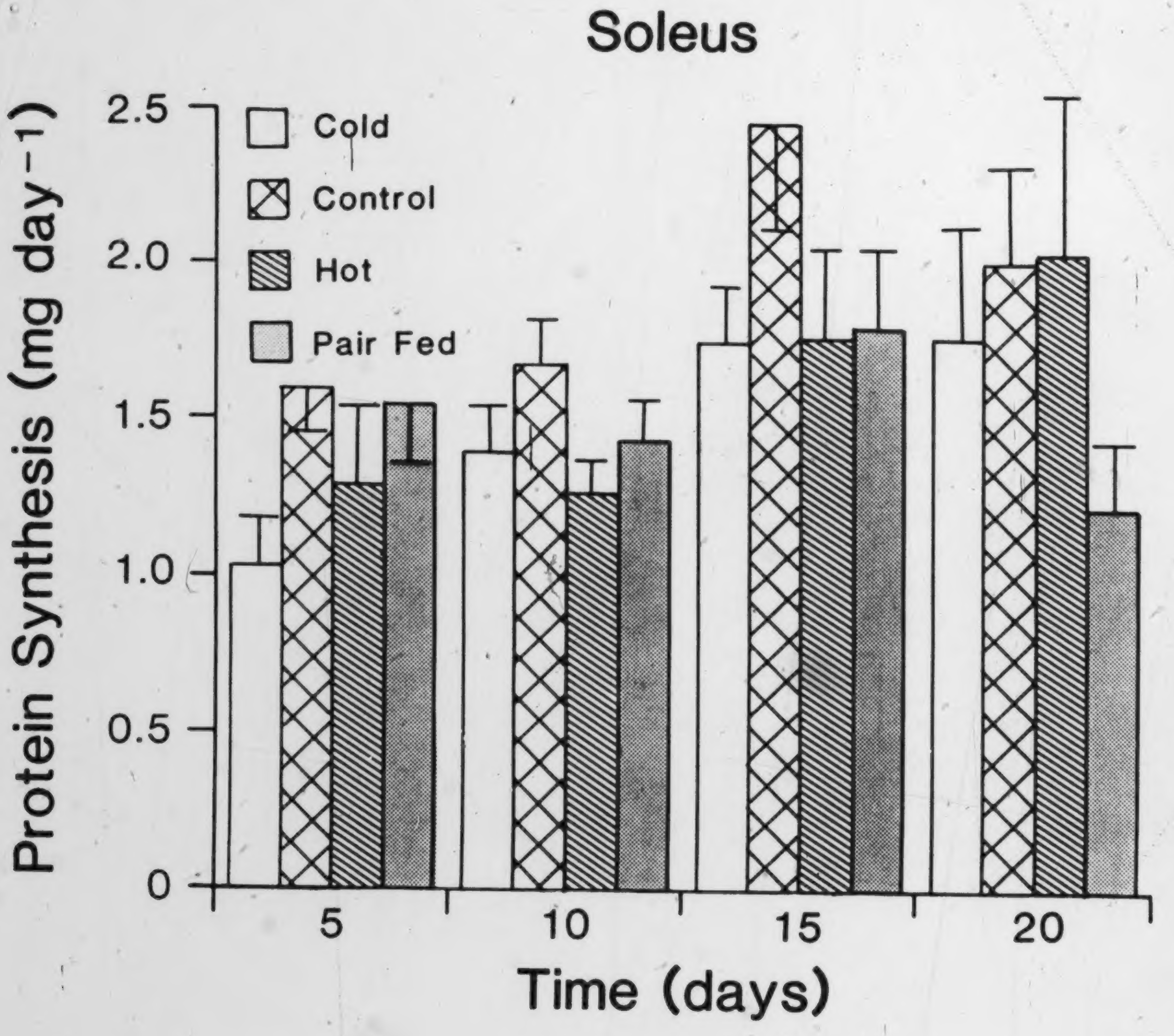

Extensor Digitorum Longus
Cold
Control
Hot
Pair Fed
Protein Synthesis (mg day^{-1})
0
0.5
1.0
1.5
2.0
2.5
5
10
15
20
Time (days)

Figure III-7.—Absolute rate of protein synthesis (mg day^{-1}) in the soleus muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 3 to 6 rats. Overall, the absolute rate of protein synthesis in the soleus muscles of rats exposed to 5 C, 35 C and pair fed were significantly lower ($P<0.05$) in comparison to control rats. Bars represent SEM.



Soleus

Protein Synthesis (mg day⁻¹)
2.5
2.0
1.5
1.0
0.5
0
□ Cold
☒ Control
▨ Hot
▩ Pair Fed
5
10
15
20
Time (days)

Figure III-8. Absolute rate of protein synthesis (mg day^{-1}) in the gastrocnemius muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 4 to 6 rats. Overall, the absolute rate of protein synthesis in the gastrocnemius muscles of rats exposed to 5 C, 35 C and pair fed were significantly lower ($P<0.05$) in comparison to control rats. Bars represent SEM.

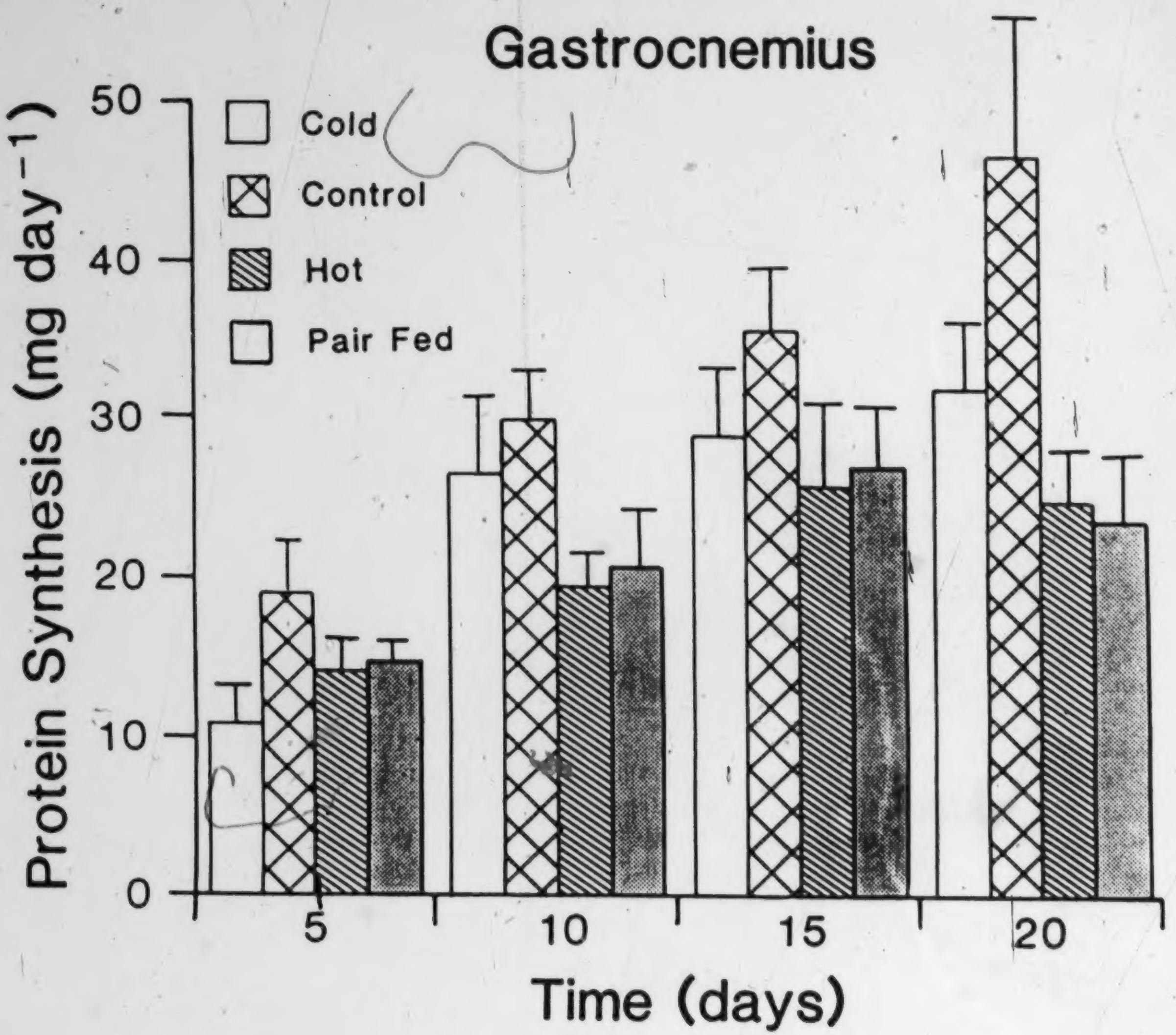

Gastrocnemius
Protein Synthesis (mg day^{-1})
0
10
20
30
40
50
Cold
Control
Hot
Pair Fed
5
10
15
20
Time (days)

Figure III-9. Absolute rate of protein synthesis (mg day^{-1}) in the heart in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 4 to 6 rats. Overall, the absolute rate of protein synthesis in the hearts of rats exposed to 5 C was significantly higher ($P<0.05$) in comparison to control rats. The absolute rate of protein synthesis in the hearts of rats exposed to 35 C and pair fed were significantly lower ($P<0.05$) than that of control rats. Bars represent SEM.

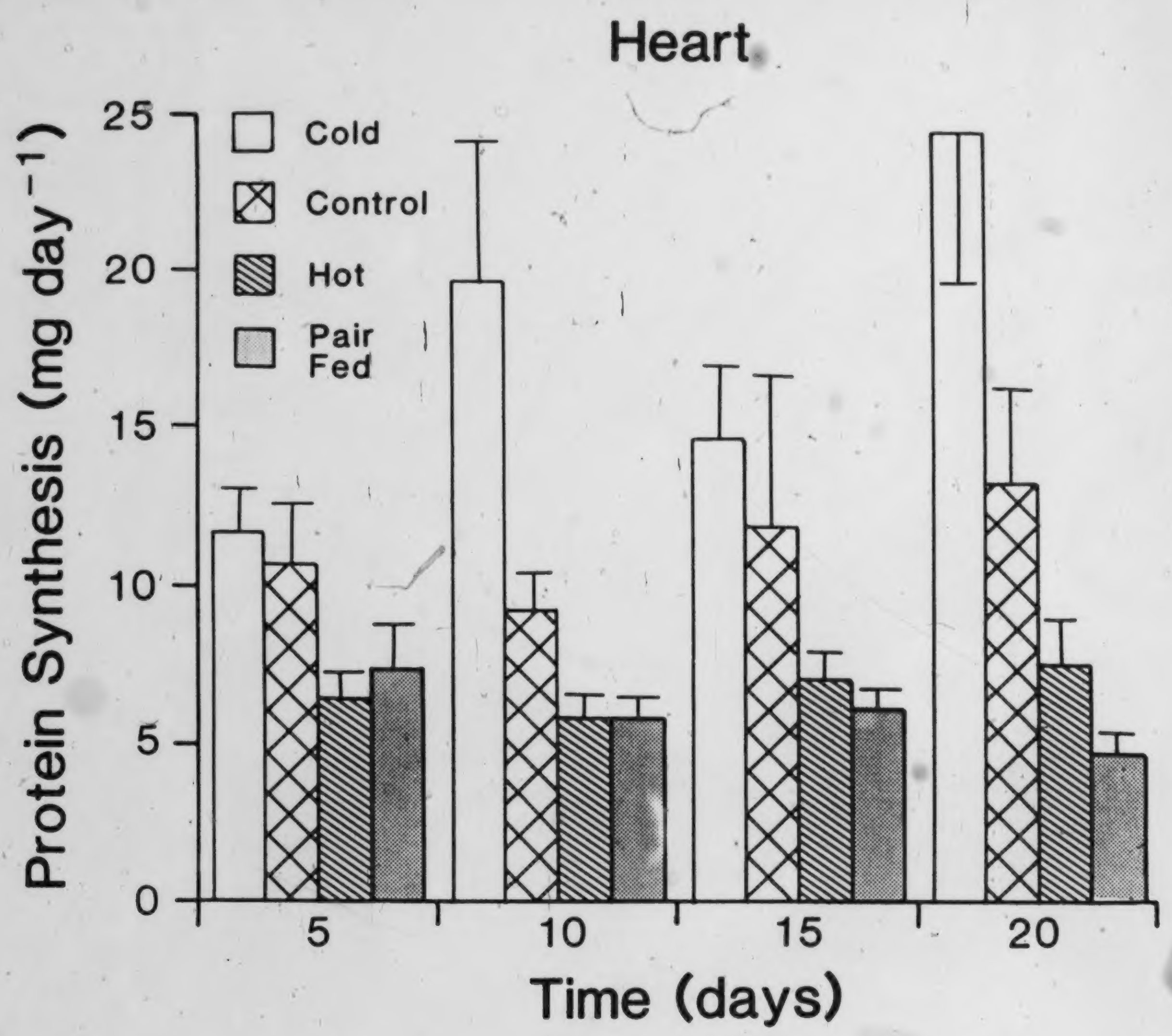

Heart
Protein Synthesis (mg day^{-1})
0
5
10
15
20
25
Cold
Control
Hot
Pair Fed
5
10
15
20
Time (days)

Figure III-10. Absolute rate of protein synthesis (mg day^{-1}) in the liver in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 3 to 5 rats. Overall, the absolute rate of protein synthesis in the liver of rats exposed to 5 C was significantly higher ($P<0.05$) than that of rats exposed to 35 C or pair fed. Bars represent SEM.

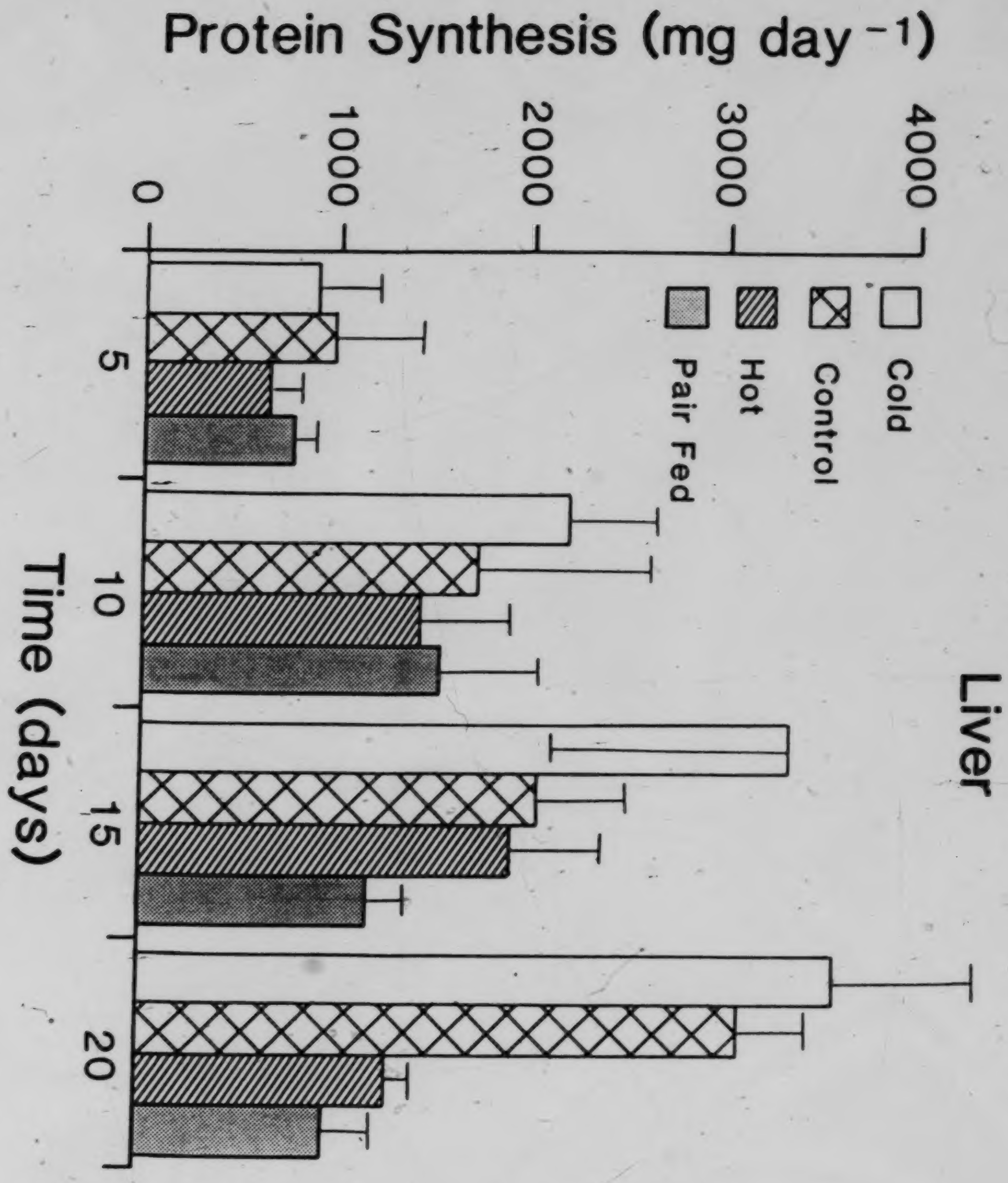

Liver
Protein Synthesis (mg day $^{-1}$)
0
1000
2000
3000
4000
☐ Cold
☒ Control
▨ Hot
▨ Pair Fed
5
10
15
20
Time (days)

Table III-2. Fractional rate of protein degradation (% day^{-1}) in tissues from rats.

Tissue		Time (days)			
		5	10	15	20
Extensor Digitorum Longus	Cold	0.11	2.38	2.64	3.09
	Control	2.28	2.61	3.95	2.60
	Hot	3.04	2.56	3.14	3.20
	Pair Fed	2.89	1.96	2.65	2.52
Soleus	Cold	5.09	6.26	5.80	5.02
	Control	5.99	4.87	5.96	3.25
	Hot	4.28	3.08	3.03	1.98
	Pair Fed	6.09	4.45	3.61	5.92
Gastrocnemius	Cold	4.94	9.09	6.96	8.12
	Control	5.04	7.50	5.25	8.42
	Hot	4.43	7.00	6.77	5.59
	Pair Fed	4.78	7.41	7.44	5.96
Heart	Cold	6.86	6.14	5.70	8.56
	Control	6.61	6.03	7.41	5.50
	Hot	6.41	5.83	4.99	6.17
	Pair Fed	8.51	4.88	5.25	4.61
Liver	Cold	169.9	187.1	201.3	164.4
	Control	123.8	175.6	143.8	138.8
	Hot	115.6	200.4	186.6	189.7
	Pair Fed	146.4	210.7	158.6	124.4

[a] mean values from 3-6 rats.

Figure III-11. Absolute rate of protein degradation (mg day^{-1}) in the extensor digitorum longus muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 3 to 5 rats.

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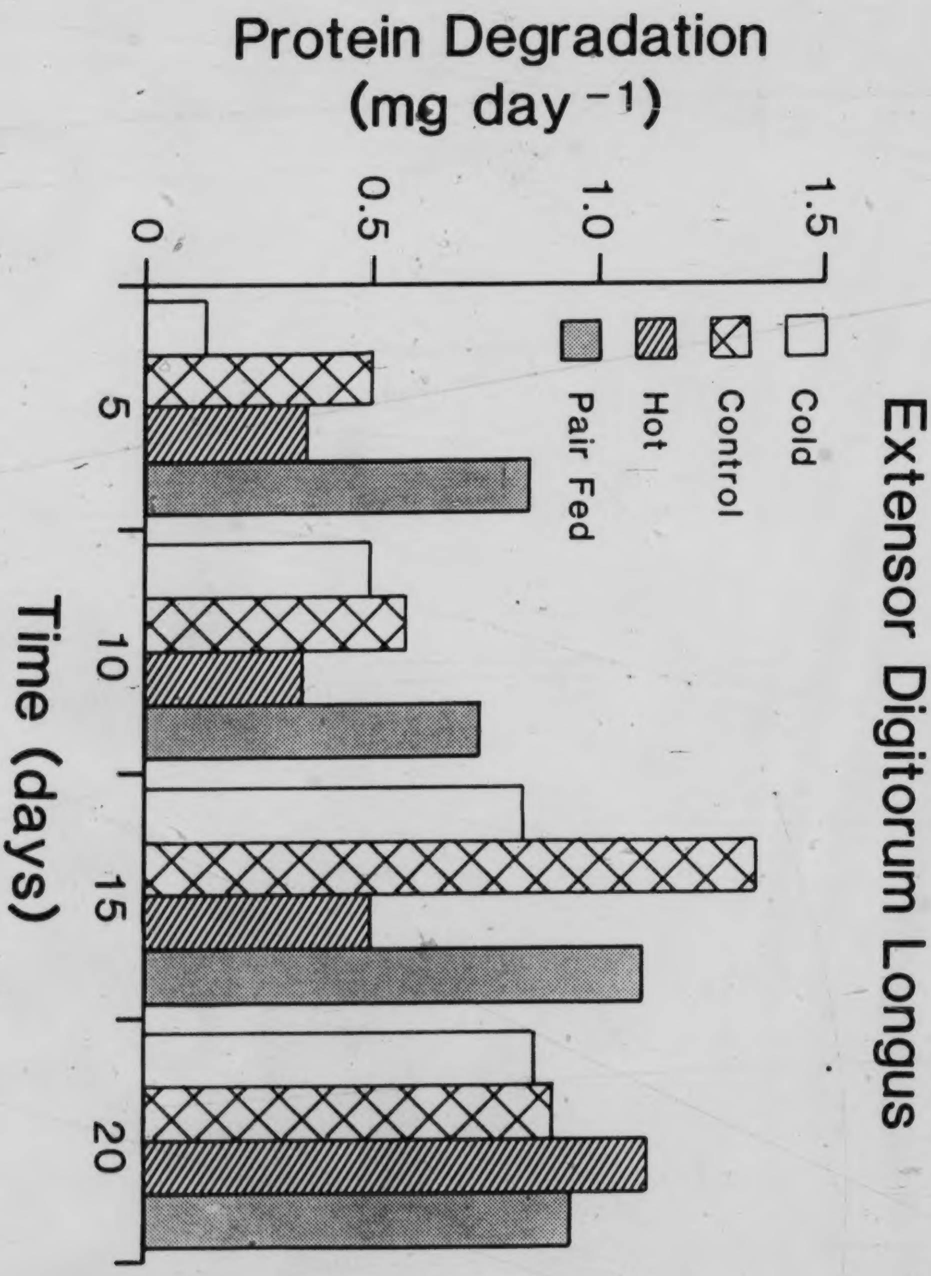

Extensor Digitorum Longus
Protein Degradation (mg day^{-1})
0
0.5
1.0
1.5
☐ Cold
☒ Control
▨ Hot
▩ Pair Fed
5
10
15
20
Time (days)

Figure III-12. Absolute rate of protein degradation (mg day^{-1}) in the soleus muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean are values from 3 to 6 rats.

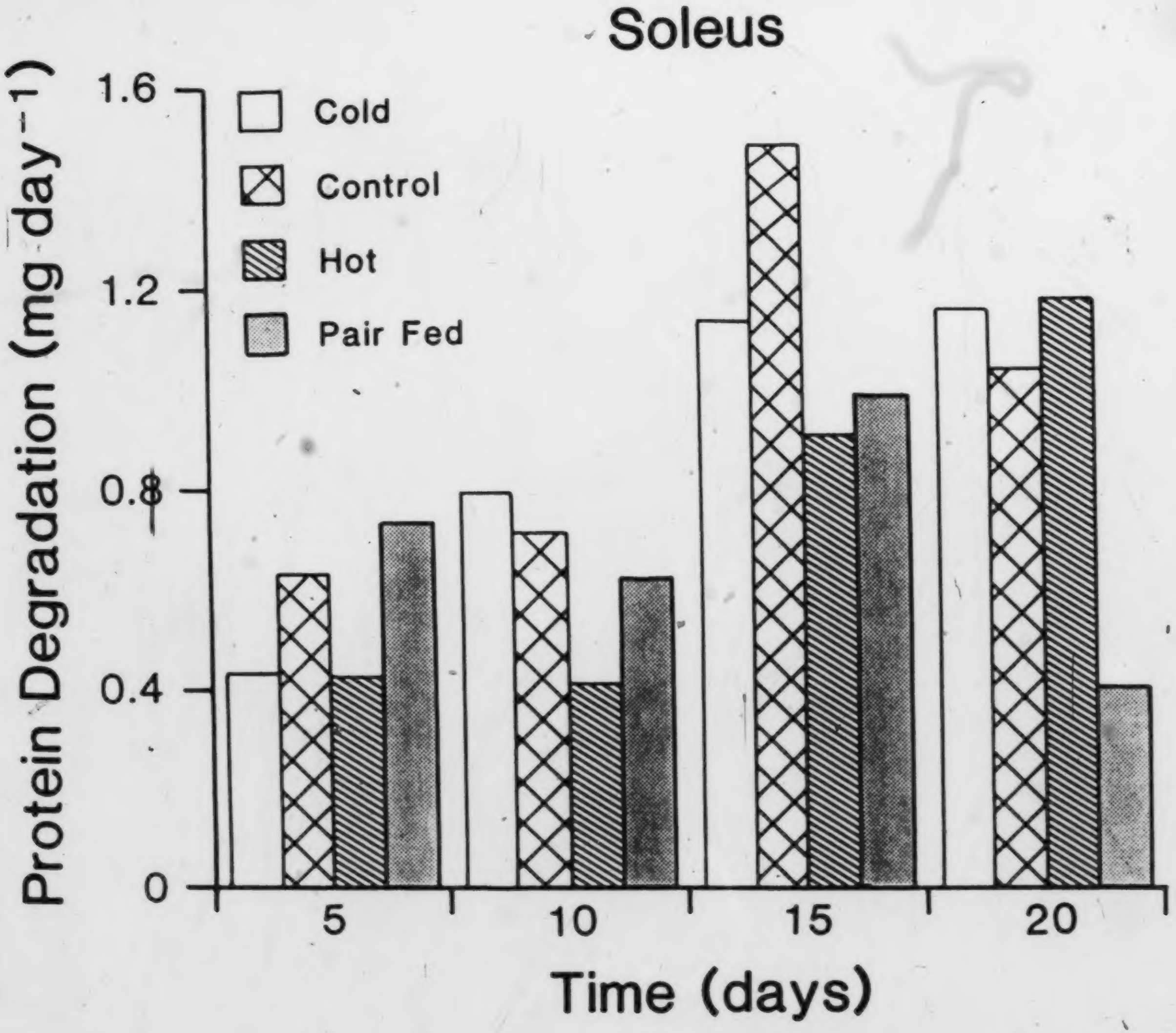

Soleus
Protein Degradation (mg day^{-1})
1.6
1.2
0.8
0.4
0
□ Cold
☒ Control
▨ Hot
▩ Pair Fed
5
10
15
20
Time (days)

Figure III-13. Absolute rate of protein degradation (mg day^{-1}) in the gastrocnemius muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 4 to 6 rats.

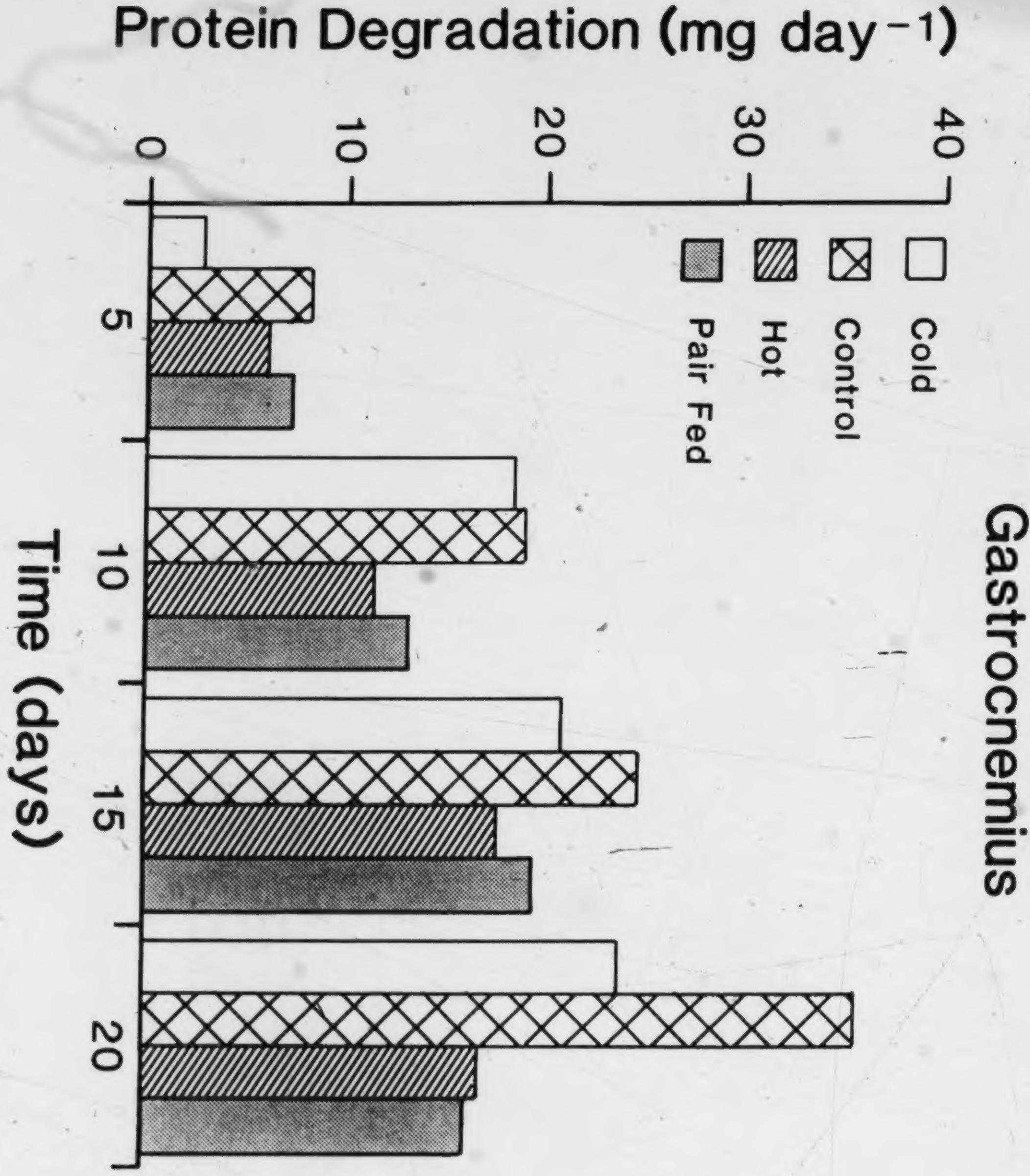
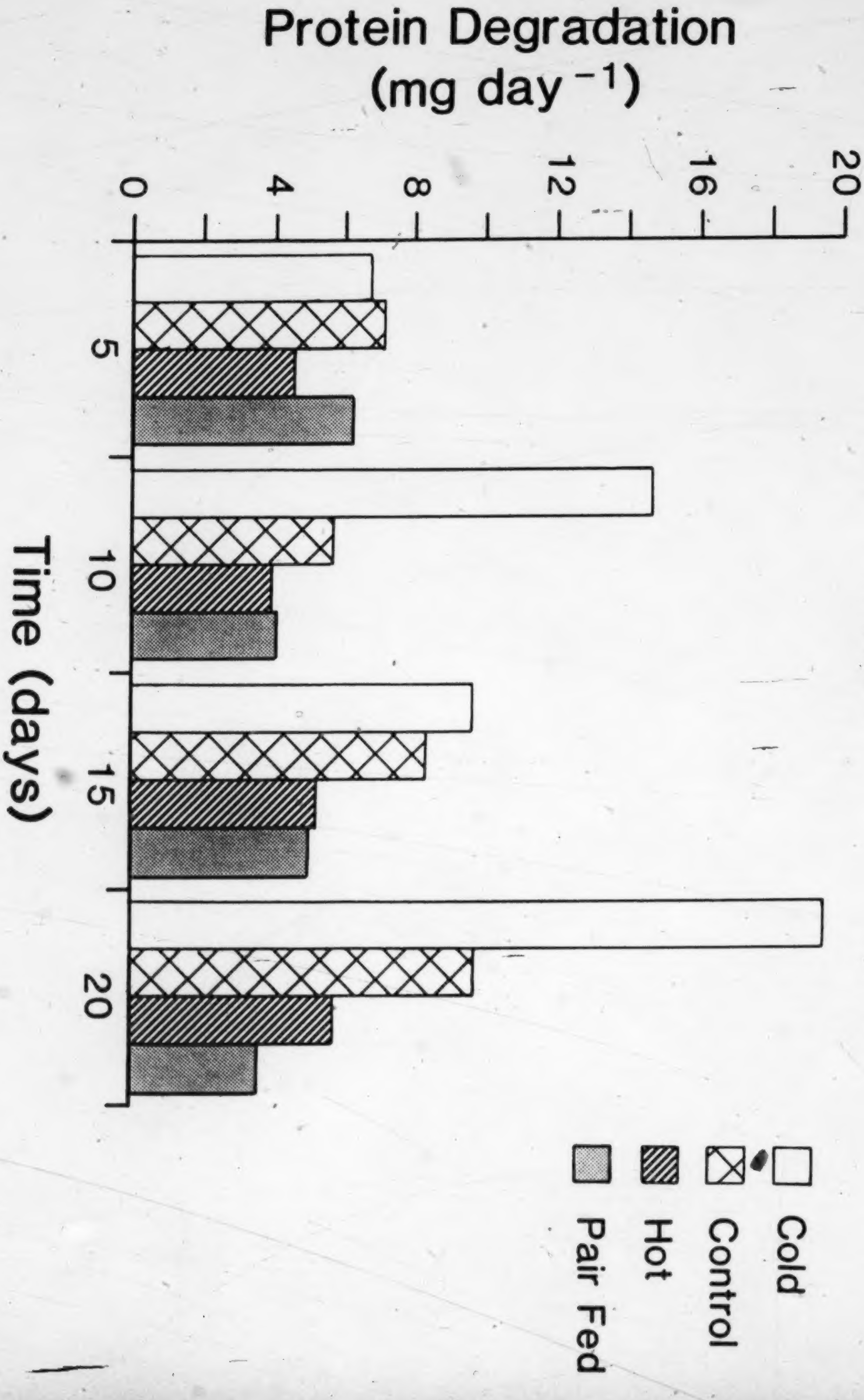

Gastrocnemius
Protein Degradation (mg day⁻¹)
0
10
20
30
40
Cold
Control
Hot
Pair Fed
5
10
15
20
Time (days)

Figure III-14. Absolute rate of protein degradation (mg day^{-1}) in the heart in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 4 to 6 rats.



Heart
Protein Degradation (mg day^{-1})
0
4
8
12
16
20
5
10
15
20
Time (days)
Cold
Control
Hot
Pair Fed

Figure III-15. Absolute rate of protein degradation (mg day^{-1}) in the liver in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 4 to 6 rats.

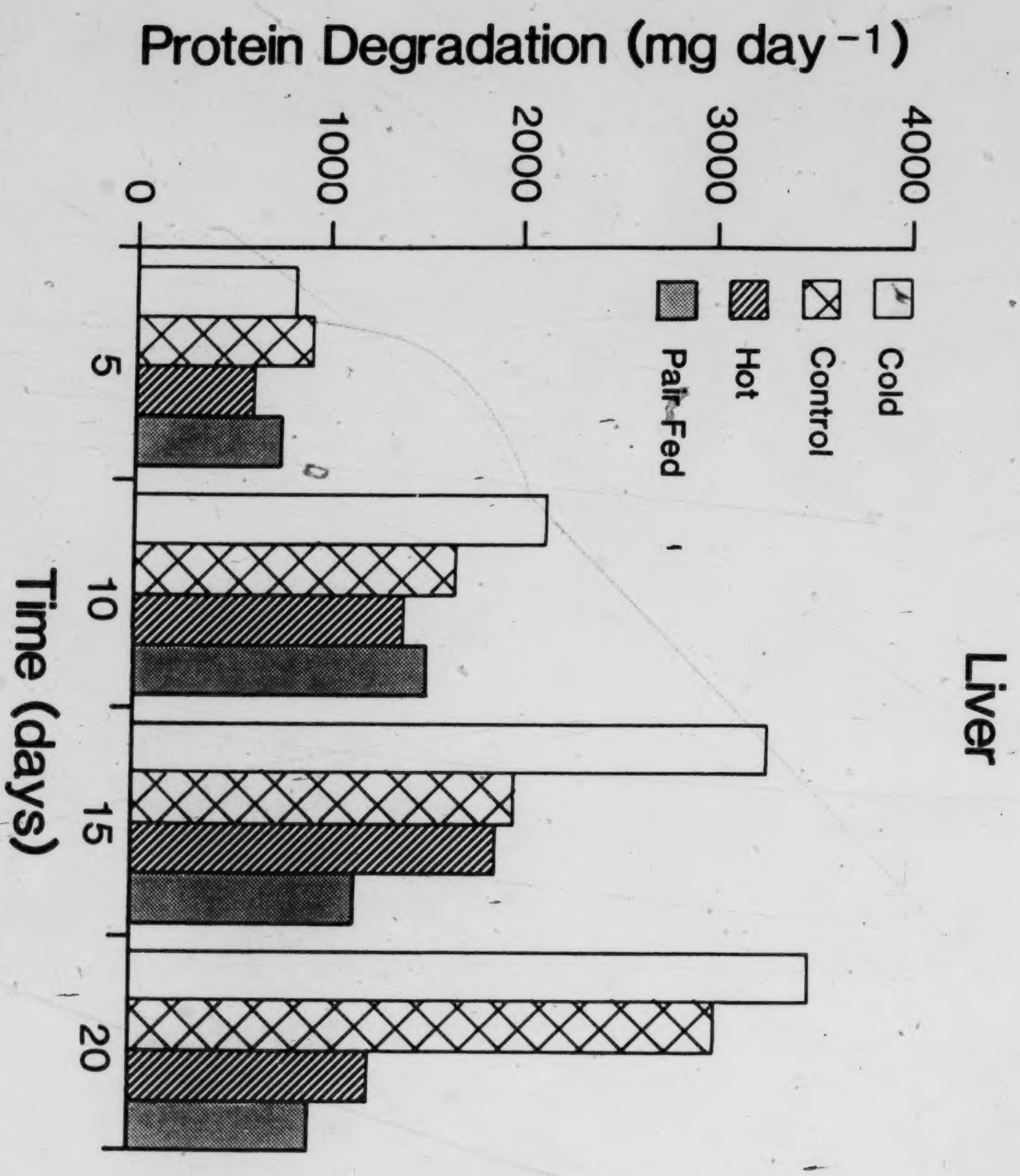
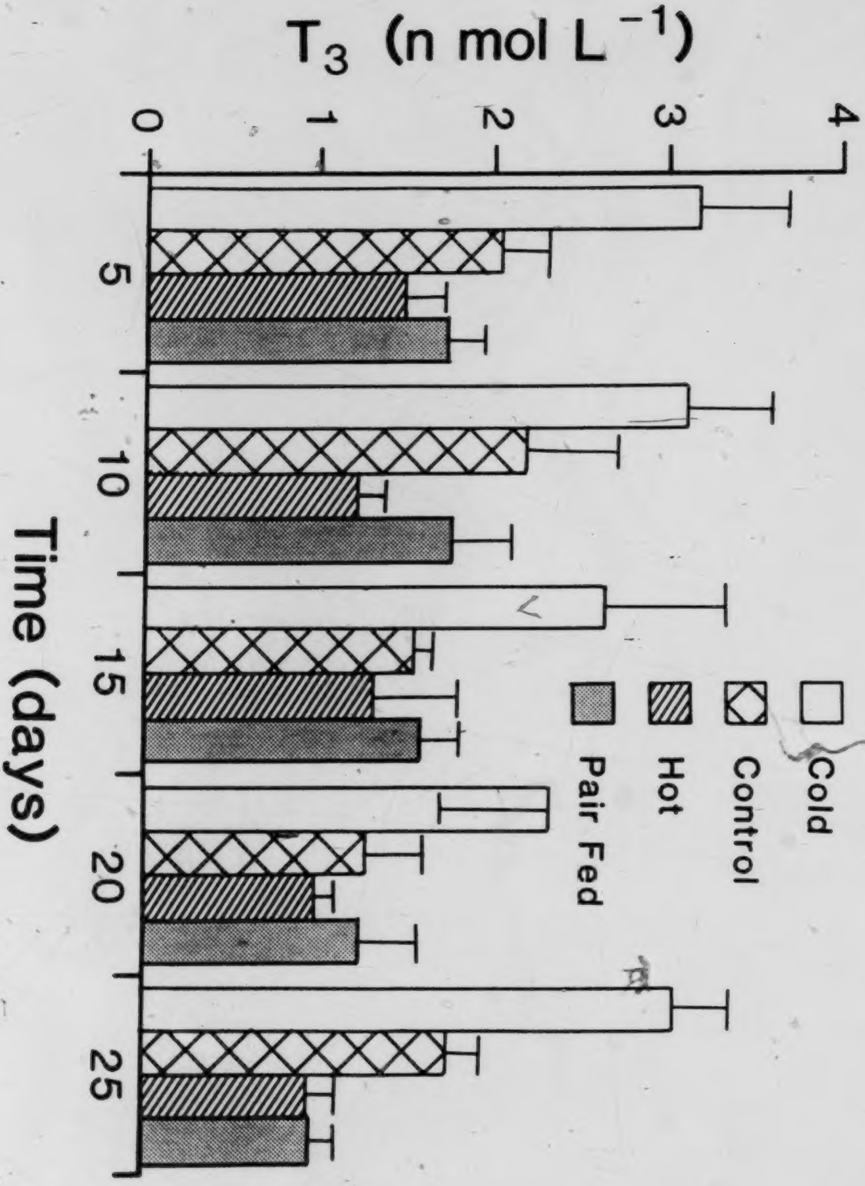

Liver
Protein Degradation (mg day $^{-1}$)
0
1000
2000
3000
4000
Cold
Control
Hot
Pair-Fed
5
10
15
20
Time (days)

Figure III-16. Plasma tri-iodothyronine concentration in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature. Mean values are from 5 to 6 rats. Bars represent SEM.


T3 (n mol L −1)
0
1
2
3
4
5
10
15
20
25
Time (days)
Cold
Control
Hot
Pair Fed

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IV. GENERAL DISCUSSION AND CONCLUSIONS

Although it is important to determine the roles that metabolites and hormones have in regulating protein turnover, it is also important to determine the changes in protein turnover in animals exposed to various stressors. The effects of stressors such as exercise (Booth and Watson 1985; Mohan et al. 1985), restraint (Goldspink et al. 1983; Preedy and Garlick 1984) and starvation (El Haj et al. 1986; Golden et al.; 1977) on the rate of protein turnover have been reported. However, few experiments have been designed to determine the effect of changes in environmental temperature on protein turnover in vivo. Therefore, the objective of this thesis was to determine the effect of elevated and reduced environmental temperatures on protein turnover in several tissues of the laboratory rat.

To help accomplish this objective, improvements were made to the method of Garlick et al. (1980) for measuring the rate of protein synthesis in vivo. Garlick et al. (1980) used a spectrofluormetric method orginally developed by Suzuki and Yagi (1976) for the estimation of free and protein bound phenylalanine. However, the ninhydrin derivative of the ß-phenylethylamine formed in this method is highly unstable in the presence of light and heat. To avoid these problems, an automated high-performance liquid chromatographic method for the analysis of ß-phenylethylamine was developed as outlined in Chapter II. The method is extremely sensitive and eliminates the need to perform time consuming column extractions when isolating ß-phenylethylamine. The organic extraction of ß-phenylethylamine can be carefully monitored for the presence of contaminants. Since the analysis time is 10.3 min sample^{-1} the addition of an autosampler to an HPLC system enables one to analyze 90-100 samples day^{-1}. It was demonstrated with this method that it is necessary to correct for endogenous free ß-phenylethylamine in the liver, a factor that was not considered by previous researchers (Garlick et al. 1980; Reeds et al. 1982; Sampson et al. 1984;) using the ninhydrin method.

Previous researchers (Garlick et al. 1980; Sampson et al. 1984) examining the rate of protein turnover in live rats administered the flooding dose of ^{3}H-phenylalanine via a lateral tail vein. This procedure can be somewhat time consuming and difficult to perform due to the small diameter of the tail vein. The problems associated with using this injection route have prompted some researchers (Sampson et al 1984) to use anaethesia to subdue the rat during injection. In addition, both the immobilization and stress associated with this type of injection may alter the rate of protein synthesis (Preedy and Garlick 1984). In an attempt to minimize these problems, a flooding intraperitoneal dose of ^{3}H-phenylalanine was administered in the present study.

In order to properly estimate the rate of protein synthesis it is necessary to determine the tissue free phenylalanine specific radioactivity-time curve between the time of isotope administration and death (Waterlow et al. 1978). Therefore, the objective of the first experiment (Appendix 1) was to characterize the specific radioactivity-time course changes of tissue free phenylalanine in skeletal muscle and liver in rats housed at three different environmental temperatures following an intraperitoneal injection of a flooding dose of phenylalanine.

The specific radioactivity of free phenylalanine in the skeletal muscles reached a plateau within 5 min, which was maintained for at least 20 min. The specific radioactivity of free phenylalanine in the liver was more variable and declined in contrast to skeletal muscle. Garlick et al. (1980) found a similar plateau in skeletal muscle free phenylalanine specific radioactivity when rats were given an injection via the lateral tail vein. The present study also indicated that the free phenylalanine specific radioactivity differed between muscles and between muscles and the liver. The free phenylalanine specific radioactivity of the liver was substantially lower than that of the three skeletal muscles studied. Therefore, extrapolation of free phenylalanine specific radioactivity values between tissues would produce errors in the estimate of protein synthesis. Reeds et al. (1982) also found that the free phenylalanine specific radioactivity of liver was lower than that of muscle after rats were given an intraperitoneal flooding dose of ^{3}H-phenylalanine. In contrast, Garlick et al. (1980) found that the free phenylalanine specific radioactivity of liver was indistinguishable from that of the gastrocnemius muscle following an intravenous injection of a flooding dose of ^{3}H-phenylalanine. The reason for this discrepancy is unknown but the lower free phenylalanine specific radioactivity of liver is likely due to the greater rate of protein turnover in this tissue releasing more unlabeled free phenylalanine to decrease the free phenylalanine specific radioactivity. It is unkown why Garlick et al. (1980) did not find a similar dilution of the specific radioactivity of liver when a flooding dose of ^{3}H-phenylalanine was administered intravenously.

The free phenylalanine specific radioactivity of rat tissues at 35 C was higher than that of tissues from rats housed at 25 or 5 C. A reduction in the rate of protein degradation in the skeletal muscle of rats exposed to hot temperature would reduce the amount of unlabled free phenylalanine released from protein, resulting in a higher free phenylalanine specific radioactivity.

This study demonstrated that a suitable plateau in the free phenylalanine specific radioactivity for the measurement of protein synthesis in skeletal muscle was acheived after an intraperitoneal injection of ^{3}H-phenylalanine. The free phenylalanine specific radioactivity of liver was more variable and declined at a faster rate. The values calculated for the fractional rate of protein synthesis in muscle and liver correspond to those previously reported in the literature.

Several researchers have demonstrated that exposure of animals to cold (Beaton 1963; Bavetta and Nimni, 1964) or hot (Hale and Mefferd 1963; Beisel et al. 1968) temperatures leads to an increase in urinary nitrogen excretion. Few researchers have examined the effect that increased or decreased environmental temperatures have on protein turnover in different tissues. Therefore, the purpose of the second experiment (Chapter III) was to determine the effect of environmental temperature on protein turnover in several tissues of the laboratory rat.

The results from the second experiment suggest that as a whole, the absolute and fractional rates of protein synthesis and degradation are reduced in skeletal muscle by exposure to both cold and hot temperatures. In contrast, the absolute and fractional rates of protein synthesis and degradation in the heart were both increased by cold exposure and decreased by heat exposure. The fractional rate of protein synthesis and degradation in the liver of rats tended to be increased by both cold and heat exposure. The absolute rate of protein synthesis and degradation in the liver was increased and decreased, respectively by cold exposure while heat exposure caused a decrease in both the absolute rate of protein synthesis and degradation.

Millward et al. (1983) reported that there was a nonsignificant increase in the fractional rate of protein synthesis and degradation in the gastrocnemius muscle of cold exposed rats. Millward et al. (1983) attributed these trends in the rate of protein turnover in the gastrocnemius muscle to elevated plasma levels of T_3 during cold exposure. In the present study plasma concentrations of T_3 were increased in rats by cold exposure and decreased by heat exposure. However, the absolute rate of protein synthesis and degradation in skeletal muscle decreased in rats exposed to 5 and 35 C. Therefore, changes in protein turnover in skeletal muscles during cold exposure may be regulated by other hormones such as insulin (Smith et al. 1983). The reduced plasma concentration of T_3 may be partially responsible for the decline in protein synthesis and degradation in rats exposed to 35 C.

The changes in protein synthesis and degradation in the heart muscle may be related to changes in the amount of contractile work performed by the heart of rats exposed to different temperatures. Previous researchers have demonstrated that increased contractile work in skeletal muscle results in an increase in protein synthesis (Smith et al. 1983; Palmer et al. 1981) and an increase in protein degradation (Laurent et al. 1978). Cold exposure leads to an increase in heart rate (Bell et al. 1975) which in turn may cause an increase in the rate of protein synthesis and degradation. Heat exposure leads to a decrease in heart rate (Cassuto 1968), possibly producing a decline in the rate of protein synthesis and degradation. However, the similarity in the rates of protein synthesis and degradation in the hearts of rats pair fed compared to rats exposed to 35 C suggests that alterations in cardiac protein turnover are partially related to feed intake.

The alterations in the absolute rate of protein synthesis and degradation in the liver resulting from temperature exposure appear to be related to feed intake. The greater feed intake of cold-exposed rats would cause an increase in the metabolic activity of the liver.

The increased metabolic activity of the liver may result in accelerated rates of protein synthesis and degradation. The reduction in the absolute rates of protein synthesis and degradation in the liver of rats exposed to 35 C reflects the lower amount of protein being deposited in the liver. The increase in the fractional rates of protein synthesis and degradation in the liver of rats exposed to 35 C indicates that the rate of turnover in the liver is accelerated by heat exposure.

In conclusion, the accelerated urinary nitrogen excretion during cold exposure may result from an increase in feed intake and an increased rate of protein turnover in the liver and heart. The increase in urinary nitrogen excretion during heat exposure may result from a heightened rate of protein turnover in the liver. The present study demonstrated that the absolute rate of protein synthesis and degradation in skeletal muscle was decreased during cold or heat exposure.

The effect of environmental temperature on protein turnover in tissues is a relevant topic to animal production in both temperate and tropical climates. The present study suggests that exposure to extreme cold or hot temperature may inhibit the depostion of protein in meat, while cold exposure promotes the depostiion of protein in less desirable animal products such as the heart and liver. Further research is required to determine the regulatory mechanisms responsible for eliciting the changes in the rate of protein turnover in these tissues as a result of high or low temperature exposure.

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APPENDIX 1

Tissue Fluid Specific Radioactivity In Skeletal Muscles and Liver After a Single Flooding Intraperitoneal Dose of ^{3}H Phenylalanine

A. INTRODUCTION

A number of isotopic techniques have been developed for estimating the rate of protein synthesis in various tissues in vivo, based on the rate of incorporation of labeled amino acid into tissue protein. These techniques must satisfy two important criteria in order to provide a valid estimate of protein synthesis:

1) The specific radioactivity of the precursor amino acid at the site of protein synthesis must be accurately estimated.
2) An accurate time course of precursor pool specific radioactivity between the time of isotope administration and death must be determined (Waterlow et al. 1978).

Early single injection techniques used by Lajtha et al. (1957) and Roberts and Morelos (1965) failed to satisfy these criteria. Work by Nicholas et al. (1977), Vidrich et al. (1977) and McKee et al. (1978) has provided evidence that tRNA is charged by amino acids from both intracellular and extracellular pools. Lajtha et al. (1957) and Robert and Morelos (1965) incorrectly assumed that tRNA became covalently bonded to amino acids solely from intracellular pools. Therefore, these earlier studies (Lajtha et al. 1957; Roberts and Morelos 1965) failed to accurately estimate the specific radioactivity of aminoacyl-tRNA at the site of protein synthesis. Furthermore, the rapid decrease in intracellular specific radioactivity (Lajtha et al. 1977; Roberts and Morelos 1965) after a single injection of a radioactively labeled precursor amino acid makes it necessary to kill a large number of animals in order to determine the time-course change in specific radioactivity (Haider and Tarver 1969). In an attempt to avoid these problems, researchers have developed more suitable techniques for estimating the rate of protein synthesis in various tissues in vivo.

The problems associated with the earlier single injection techniques of Lajtha et al (1957) and Roberts and Morelos (1965) can be avoided by injecting single large doses of labeled amino acid (Henshaw et al. 1971; Dunlop et al. 1975). This single flooding dose technique produces nearly the same specific radioactivity in the extracellular, intracellular and aminoacyl-tRNA amino acid pools, thereby eliminating errors associated with choice of the precursor pool for calculations of protein synthesis (Henshaw et al. 1971). In addition the specific radioactivity in plasma and tissue stays relatively constant for some time, eliminating the need to kill large numbers of animals to establish the time-course changes for this parameter (Garlick et al. 1980; M^{c}Nurlan et al. 1979).

Techniques reported by Garlick et al. (1980) and M^{c}Nurlan et al. (1979) for the estimation of the rate of protein synthesis in various tissues in the rat involved the administration of a flooding dose of ^{3}H-phenylalanine via a lateral tail vein. This injection procedure is somewhat difficult to perform on young rats due to the relatively small diameter of the vein. Also, the stress and immobilization associated with attempting to inject young rats via the lateral tail vein alters the rate of protein turnover (Preedy and Garlick 1984). Subsequently, Reeds et al. (1982) injected a flooding dose of ^{3}H-phenylalanine intraperitoneally in an attempt to avoid the problems associated with lateral tail vein injection. Injection of ^{3}H-phenylalanine into the peritoneal cavity does not place ^{3}H-phenylalanine directly into the circulatory system. Thus, the time required for ^{3}H-phenylalanine to be absorbed into the blood stream may produce a tissue free phenylalanine specific radioactivity-time curve which requires a longer period of time to develop a plateau.

In this appendix the specific radioactivity-time course changes of tissue free phenylalanine in skeletal muscle and liver in rats housed at three different environmental temperatures given an intraperitoneal injection of ^{3}H-phenylalanine are characterized. In addition, the fractional rates of protein synthesis in the extensor digitorum longus, soleus and gastrocnemius muscles and liver were determined in rats exposed to three different environmental temperatures for a 10 d period.

B. MATERIALS AND METHODS

The sources of L-[2,6-^{3}H] phenylalanine and other chemicals have been described in Chapter II.

Sixty male Sprague-Dawley laboratory rats, averaging 46.0 ± 10 g body weight (Charles River, St. Constant, Quebec) were obtained at 21 d of age. The rats were housed in individual cages and placed in a light adjusted, temperature controlled room at 25 C and subjected to a 12 h light/dark cycle. All rats were provided with water and a purified diet containing 20% casein ad libitum as described in Table II-1. Rats were allowed to adjust to the new diet and housing conditions for a 7 d period.

After adaptation, 20 rats were transferred to a cold room at 10 C and a second group of 20 rats was placed in a hot room at 30 C. A control group of 20 rats remained in the 25 C room. Lighting conditions and the light/dark cycle of the two treatment rooms were equalized to the control room. After one day, the temperature of the cold room was lowered to 5 C while the hot room was raised to 35 C. The feed intake of all three groups was measured daily at 1100 h. Rats were exposed to their specific temperature treatments for a 10 d period.

On day 10 each rat was given an intraperitoneal injection of L-[2,6-^{3}H] phenylalanine as described in Chapter II. After injection, rats from each room were killed by cervical dislocation at four different times; 5, 10, 15 and 20 min. The soleus, extensor digitorum longus and gastrocnemius muscles and liver were immediately removed and placed in ice cold 2 N $HClO_4$ and stored in a freezer at -70 C. Tissue samples were prepared and the specific radioactivities of the free and protein bound phenylalanine were determined as reported in Chapter II. The fractional rate of protein synthesis for each tissue (K_s expressed as % day^{-1}) was determined from the formula of M^{c}Nurlan et al. 1979 and Garlick et al. 1980:

$$K_s = \frac{S_b \times 100\%}{S_i \times t}$$

where S_b and S_i are the specific radioactivities of the protein-bound and free phenylalanine, respectively, and t is the time available for label incorporation expressed in days.

All results were expressed as mean ± S.E.M. and were analyzed by one way variance analysis and SNK multiple simultaneous means comparison as described by Steele and Torrie (1980). A repeated measures test was used for a comparison of tissue free phenylalanine specific radioactivity between tissues and treatments (Winer 1971).

C. RESULTS

The daily feed intake of rats housed at the three different temperatures for 10 d was recorded from day 2 to day 9 (Figure A1-1, Table A1-1). Daily feed intake of rats housed in the cold increased continuously for 6 d and was significantly higher each day ($P<0.05$) than in the control rats throughout the trial. The feed intake of rats at 35 C remained relatively constant and was lower ($P<0.05$) than that of the control rats for each day during the trial.

Figures A1-2, 3, 4 illustrate the specific radioactivity-time curves of free phenylalanine in tissues of rats housed at the three different temperatures. In skeletal muscle the specific radioactivity of free phenylalanine reaches a plateau within 5 min which is maintained for at least 20 min. The free phenylalanine average specific radioactivity differed between all tissues ($P<0.001$). The extensor digitorum longus muscle had the highest ($P<0.001$) free phenylalanine specific radioactivity followed by the soleus and gastrocnemius muscles. The free phenylalanine average specific radioactivity of the liver was much lower ($P<0.001$) than that of the skeletal muscles. As a whole, the free phenylalanine average specific radioactivity of tissues from rats at 35 C (Figure A1-4) was higher ($P<0.001$) than that for both control rats (Figure A1-3) and rats at 5 C (Figure A1-2).

The fractional rates of protein synthesis in the extensor digitorum longus, soleus and gastrocnemius muscles and liver are shown in Figures A1-5, 6, 7, 8, respectively. The fractional rate of protein synthesis in the extensor digitorum longus muscle was not altered ($P>0.05$) when rats were exposed to cold (Mean ±S.E. 9.7 ±2.0) or hot (9.6 ± 0.5) temperatures for 10 d in comparison to control rats (10.1 ±1.0) (Figure A1-5). In the soleus muscle, protein synthesis was reduced ($P<0.05$) in rats exposed to 5 C (9.9 ±1.8) in comparison with control rats (14.9 ±0.9) (Figure A1-6). Protein synthesis in the soleus muscle of rats exposed to 35 C (10.5 ±1.0) was not decreased ($P>0.05$) but there was a trend towards a decrease. Exposure of rats to cold or hot temperature had no effect ($P>0.05$) on protein synthesis in the gastrocnemius muscle (Figure A1-7). The fractional rate of protein synthesis in the gastrocnemius muscle tended to be lower in rats exposed to 35 C (8.0 ±1.4) and 5 C (10.5 ±1.0) when compared to control rates (14.4 ±4.4). Protein synthesis in the liver tended to be higher in rats exposed to cold (176 ±28) and hot (144 ±24) temperatures than in control rats (93 ±21) (Figure A1-8). However, due to the large degree of variation within treatments these values were not ($P>0.05$) different.

D. DISCUSSION

The intravenous tail vein injection procedure originally used by M^{c}Nurlan et al. (1979) is difficult to perform on young rats. Reeds et al. (1982) encountered similar problems and were unable to administer radioisotope to Zucker rats via the tail vein. As a result, Reeds et al. (1982) and the present authors administered the radioisotope intraperitoneally. Intraperitoneal injection of radioisotope has several advantages over the tail vein procedure. Intraperitoneal injection avoids the problems associated with attempting to insert a needle into a small vein. Injection into the lower left quadrant of rats is relatively easy and does not require the precision of the tail vein injection. Because the intraperitoneal injection is easy to perform it requires less time, enabling more rats to be injected and reduces the apparent amount of stress that each rat receives. This is especially important since it has been demonstrated that immobilization and stress can alter the rates of protein synthesis in rat skeletal muscle (Watson et al. 1984; Preedy and Garlick 1984).

Garlick et al. (1980) demonstrated that there is a slow linear decline in the specific radioactivity of free phenylalanine in the tissues of the rat after an intravenous injection of ^{3}H-phenylalanine. Figures A1-2, 3, 4 illustrate that the free phenylalanine specific radioactivity in skeletal muscle remained relatively constant during the 20 min period examined after an intraperitoneal injection of ^{3}H-phenylalanine. The free phenylalanine specific radioactivity of the liver was more variable than muscle and tended to decline during the 20 min period. Garlick et al. (1980) found that the free phenylalanine specific radioactivity of liver was indistinguishable from that of the gastrocnemius muscle in rats after an intravenous injection of ^{3}H-phenylalanine. However, with the intraperitoneal injection method the free phenylalanine specific radioactivity differed significantly between all tissues (Figure A1-2, 3, 4). The present study demonstrates that the single flooding dose technique does not produce the same free phenylalanine average specific radioactivity in different tissues including different muscles and liver. Therefore, the free phenylalanine average specific radioactivity of one tissue cannot be used to estimate the free phenylalanine specific radioactivity of another type of tissue without producing errors in the estimation of protein synthesis. This makes it necessary to determine the specific radioactivity of free phenylalanine for the liver and each muscle separately. The difference in free phenylalanine specific radioactivity was much greater between the liver and muscles than it was between muscles, with the free phenylalanine specific radioactivity of liver being substantially lower than that of the muscles. Reeds et al. (1982) also found that the free phenylalanine specific radioactivity of liver was lower than the free phenylalanine specific radioactivity of muscle after rats were injected intraperitoneally with radioactive phenylalanine. The reason for the lower free phenylalanine specific radioactivity in liver is unknown. Several authors have demonstrated that the rate of protein turnover in the liver is much greater than in skeletal muscle (e.g. Garlick et al. 1975; Odedra et al. 1983). Thus, the liver releases more unlabeled phenylalanine from protein causing a dilution of the free phenylalanine specific radioactivity.

The reason why the free phenylalanine specific radioactivity in muscles from rats at 35 C was higher than in control rats or rats at 5 C is unclear. A reduction in the release of phenylalanine from tissue protein in rats at 35 C may account for the higher free phenylalanine specific radioactivity observed.

Since a plateau in muscle free phenylalanine specific radioactivity was established in each treatment, differences in free phenylalanine specific radioactivity between treatments does not effect the calculation of protein synthesis. In their original paper Garlick et al. (1980) calculated protein synthesis from the following formula:

$$K_s = \frac{S_b \times 100\%}{S_a \times t}$$

The mean specific radioactivity of free phenylalanine (S_a) was obtained by killing rats at 2 and 10 min following injection. The decline in free phenylalanine specific radioactivity in rat tissues was quite small and was shown to be less than 5 % over the 10 min incorporation period (Garlick et al. 1980). In the present study, the changes in free phenylalanine specific radioactivity in rat skeletal muscle over a 20 min period were less than those of Garlick et al. (1980). Consequently, in the present paper as in past studies (Garlick et al. 1982) a single time point (i.e. 15 min) has been used to estimate the free phenylalanine specific radioactivity. Avoiding the need to accurately describe the time course change in phenylalanine specific radioactivity reduces the number of animals required to estimate the fractional rate of protein synthesis.

It is well documented that exposure of rats to cold (Beaton 1963; Bavetta and Nimmni 1964) or hot (Mefferd et al. 1958; Payne and Jacob, 1965) environmental temperatures causes an increase and a decrease in food intake, respectively. This response makes it difficult to separate the effects of dietary intake from the effects of exposure to adverse temperatures. Many researchers have concluded that the metabolic responses to extreme temperatures, such as alterations in thyroid function, are partially due to changes in dietary intake (Himms-Haggen 1983). This problem can be partially resolved by pair feeding a control group of rats with a group in the hot (see Chapter III). This solution is not suitable for the cold since a cold group pair fed with a control group would not obtain enough energy to maintain body temperature.

The fractional rates of protein synthesis obtained for the muscles in the present study are similar to those in the literature. Goldspink

(1977) determined that the fractional rate of protein synthesis in the extensor digitorum longus muscles from young rats was 5.9 % day^{-1}. Garlick et al. (1980) estimated that the fractional rate of protein synthesis in the gastrocnemius muscles from rats was 16.9 % day^{-1}. Kelly and Goldspink (1982) found that the fractional rate of protein synthesis in the soleus muscles from rats was 14.8 % day^{-1}. In the present study the fractional rate of protein synthesis in the extensor digitorum longus, soleus and gastrocnemius muscles from control rats were 10.1, 14.3 and 14.4 % day^{-1}.

There is limited information concerning the effect of environmental temperatures on protein turnover in animal tissues. Millward et al. (1983) reported that there was a nonsignificant increase in protein synthesis in the gastrocnemius muscles from rats exposed to 4 C. In the present study, there was a nonsignificant decrease in the rate of protein synthesis in the gastrocnemius muscles from rats housed at 5 or 35 C. In the soleus muscles, protein synthesis decreased significantly in rats exposed to 5 C and tended to decrease in rats exposed to 35 C. The rate of protein synthesis in the extensor digitorum longus muscle was unchanged by treatment temperatures. Unfortunately, the data of Millward et al. (1983) originated from a preliminary study. Therefore, it is difficult to make meaningful comparisons due to the lack of experimental details. The discrepancies between the results of Millward et al. (1983) and the present study may be a consequence of different experimental conditions.

Several researchers have demonstrated that triiodothyronine (T_3) causes an increase in the rate of protein synthesis in certain rat skeletal muscles (Carter et al. 1982; Brown et al. 1981). Millward et al. (1985) proposed that an elevated level of T_3 in blood during cold exposure is responsible for the nonsignificant increase in protein synthesis in the gastrocnemius muscle. The present results do not support Millward's observations in cold exposed rats. Since Millward et al. (1983) and the present author did not reveal a significant change in protein synthesis in the gastrocnemius muscle of cold-exposed rats it is possible that the differences noted are a result of random variation. The present study indicates that stress from cold exposure can cause a significant reduction in the rate of protein synthesis in the soleus muscle.

Despite the problems encountered in estimating the free phenylalanine specific radioactivity, protein synthesis values in the liver when calculated correspond with values in the literature. Garlick et al. (1982) determined that the fractional rate of protein synthesis in the livers from young male rats was 86 % day^{-1}. In an earlier study, M^{c}Nurlan and Garlick (1980) estimated that the fractional rate of protein synthesis in rat liver was 105 % day^{-1}. Sampson et al. (1984) found that the fractional rate of protein synthesis in the liver during lactation in rats was 70 % day^{-1}. In the present study the fractional rate of protein synthesis in livers from young male rats averaged 92 % day^{-1}. The rate of protein synthesis in livers from heat-exposed and cold-exposed rats increased to 143 and 176 % day^{-1}, respectively. This change indicates that stress from environmental temperatures may accelerate the rate of protein synthesis in the liver. Other researchers have also found that stress can increase the rate of protein synthesis in the liver. For example, Garlick et al. (1975) found that the fractional rate of protein synthesis in the liver was increased significantly in malnourished rats.

This experiment indicates that it is difficult to separate the effects of exposure to extreme environmental temperature from changes in dietary intake. In future experiments part of this problem could be eliminated by pair feeding. The intraperitoneal injection of radioisotope is suitable for measuring protein synthesis in rat muscle, but results from liver are extremely variable. However, protein synthesis rates from the liver and muscle do coincide with values found in the literature. Further research is required to determine the relationship between temperature acclamation and protein turnover.

Figure A1-1. Daily feed intake values of rats housed at three different temperatures; cold (5 C), control (25 C) and hot (35 C). Mean values are from 30 to 34 rats. Feed intake of rats housed in the cold and in the hot environments were significantly higher ($P<0.05$) and significantly lower ($P<0.05$), respectively, than in rats housed in the control environment.

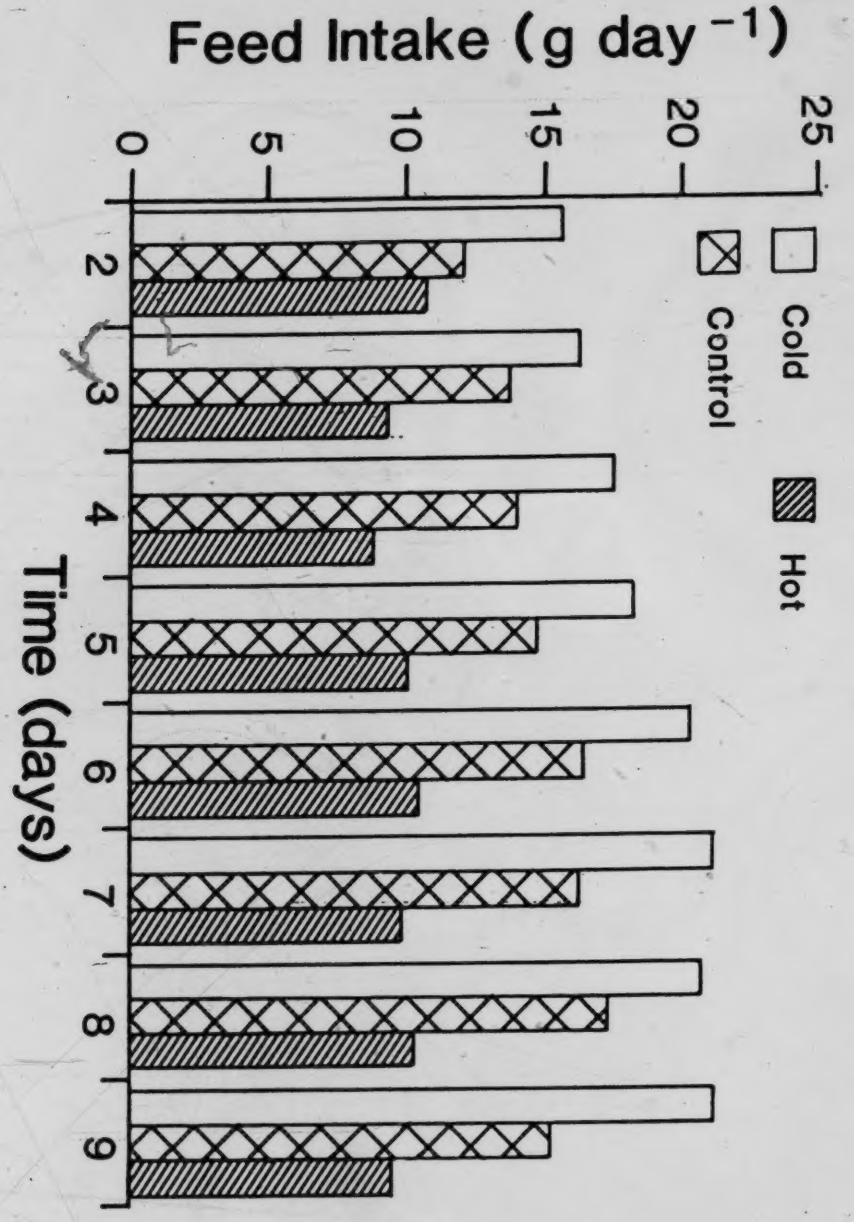

Feed Intake (g day $^{-1}$)
0
5
10
15
20
25
Cold
Control
Hot
2
3
4
5
6
7
8
9
Time (days)

Table A1-1. Feed intake of rats (g day^{-1}) housed at three different temperatures cold (5 C), control (25 C) and hot (35 C).@

Time (days)	Group Cold	 Hot	 Control
2	12.17 A (.34)	10.80 B (.33)	15.76 C (.36)
3	16.43 A (.59)	9.41 B (.37)	13.83 C (.49)
4	17.60 A (.56)	8.90 B (.28)	14.11 C (.53)
5	18.37 A (.57)	10.22 B (.27)	14.49 C (.74)
6	20.41 A (.60)	10.57 B (.44)	16.53 C (.80)
7	21.25 A (.49)	9.98 B (.38)	16.41 C (.59)
8	20.81 A (.80)	10.45 B (.28)	17.39 C (.62)
9	21.26 A (.80)	9.6 B (.28)	15.33 C (.59)

@ mean values from 30 to 34 rats

( ) standard error of the mean

A,B,C values are significantly different from each other at $P<0.05$.

Figure A1-2. Time course changes in specific radioactivity of free phenylalanine in extensor digitorum longus (-□-□-□-), soleus (-✱-✱-) and gastrocnemius (-▲-▲-) muscles and liver (-⌧-⌧-) of rats housed at 5 C. Mean values are from 3-5 rats.

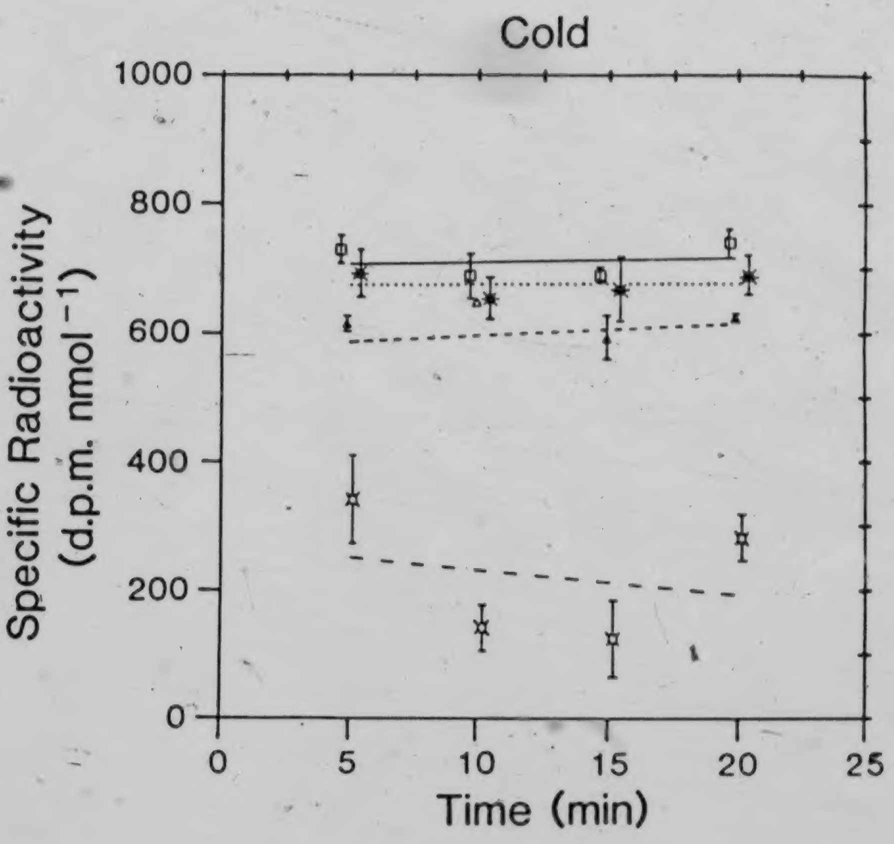

Cold
Specific Radioactivity
(d.p.m. nmol⁻¹)
1000
800
600
400
200
0
0
5
10
15
20
25
Time (min)

Figure A1-3. Time course changes in specific radioactivity of free phenylalanine in extensor digitorum longus (-■-■-■-), soleus (-✱-✱-) and gastrocnemius (-▲-▲-) muscles and liver (-⌧-⌧-) of rats housed at 25 C. Mean values are from 3-5 rats.

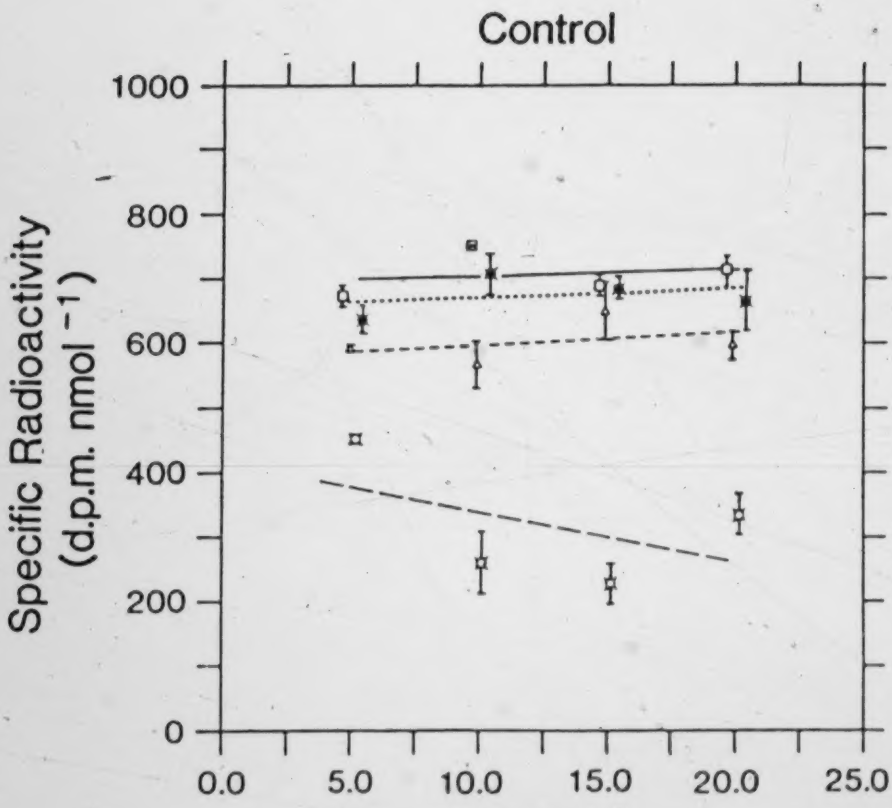

Control
1000
800
600
400
200
0
Specific Radioactivity (d.p.m. nmol ⁻¹)
0.0
5.0
10.0
15.0
20.0
25.0
Time (min)

Figure A1-4. Time course changes in specific radioactivity of free phenylalanine in extensor digitorum longus (-⊟-⊟-⊟-), soleus (-✱-✱-) and gastrocnemius (-△-△-) muscles and liver (-☆-☆-) of rats housed at 35 C. Mean values from 3-5 rats.

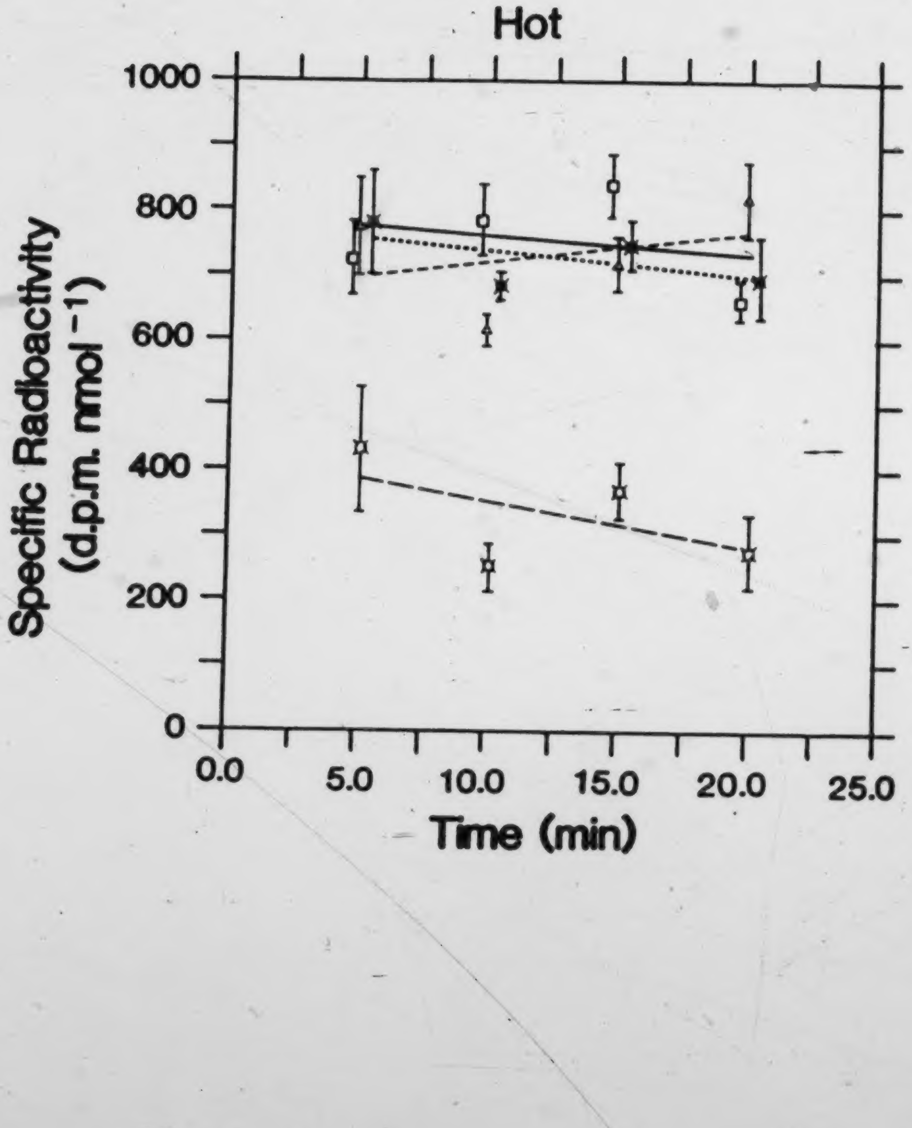
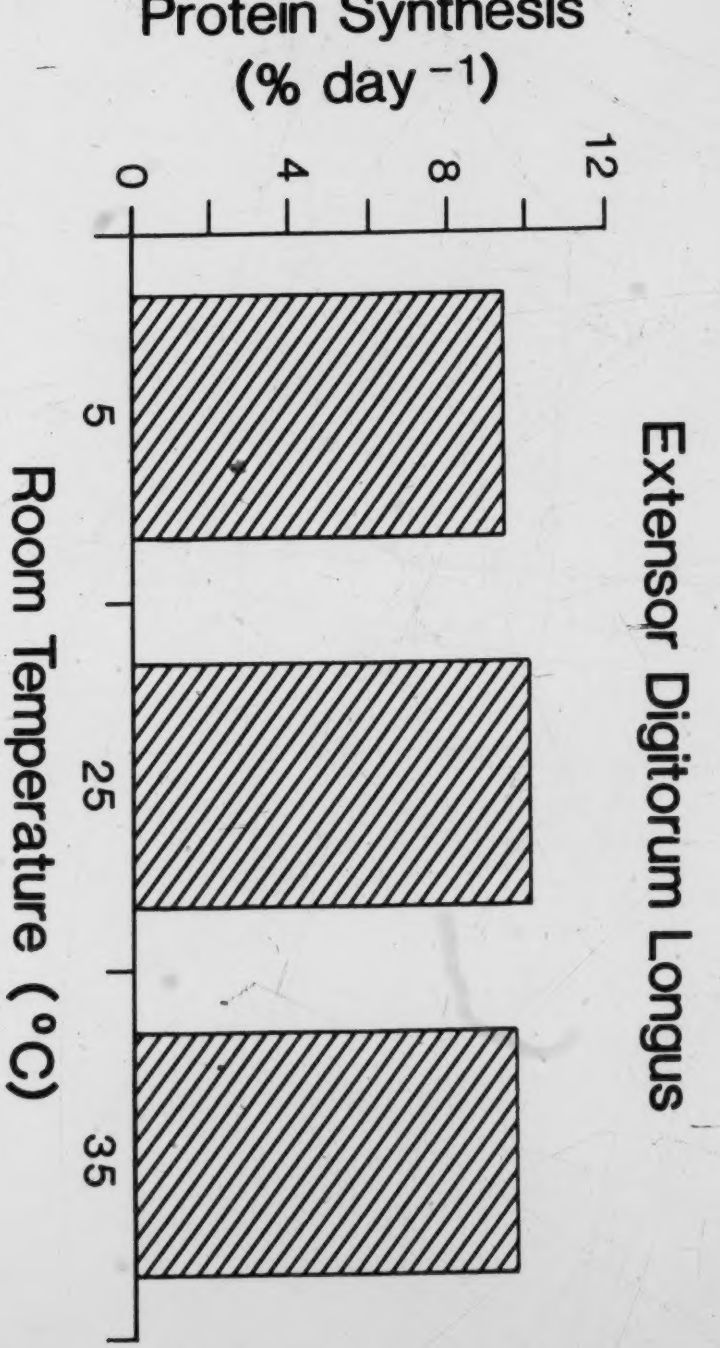

Hot
Specific Radioactivity
(d.p.m. nmol^{-1})
1000
800
600
400
200
0
0.0
5.0
10.0
15.0
20.0
25.0
Time (min)

Figure A1-5. Fractional rate of protein synthesis (% day^{-1}) in the extensor digitorum longus muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Mean values from 9 to 10 rats.



Extensor Digitorum Longus
Protein Synthesis
(% day $^{-1}$)
0
4
8
12
5
25
35
Room Temperature (°C)

Figure A1-6. Fractional rate of protein synthesis (% day^{-1}) in the soleus muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Mean values from 9 to 10 rats. The fractional rate of protein synthesis in the soleus muscles of rats exposed to 5 C were lower ($P<0.05$) in comparison to control rats.

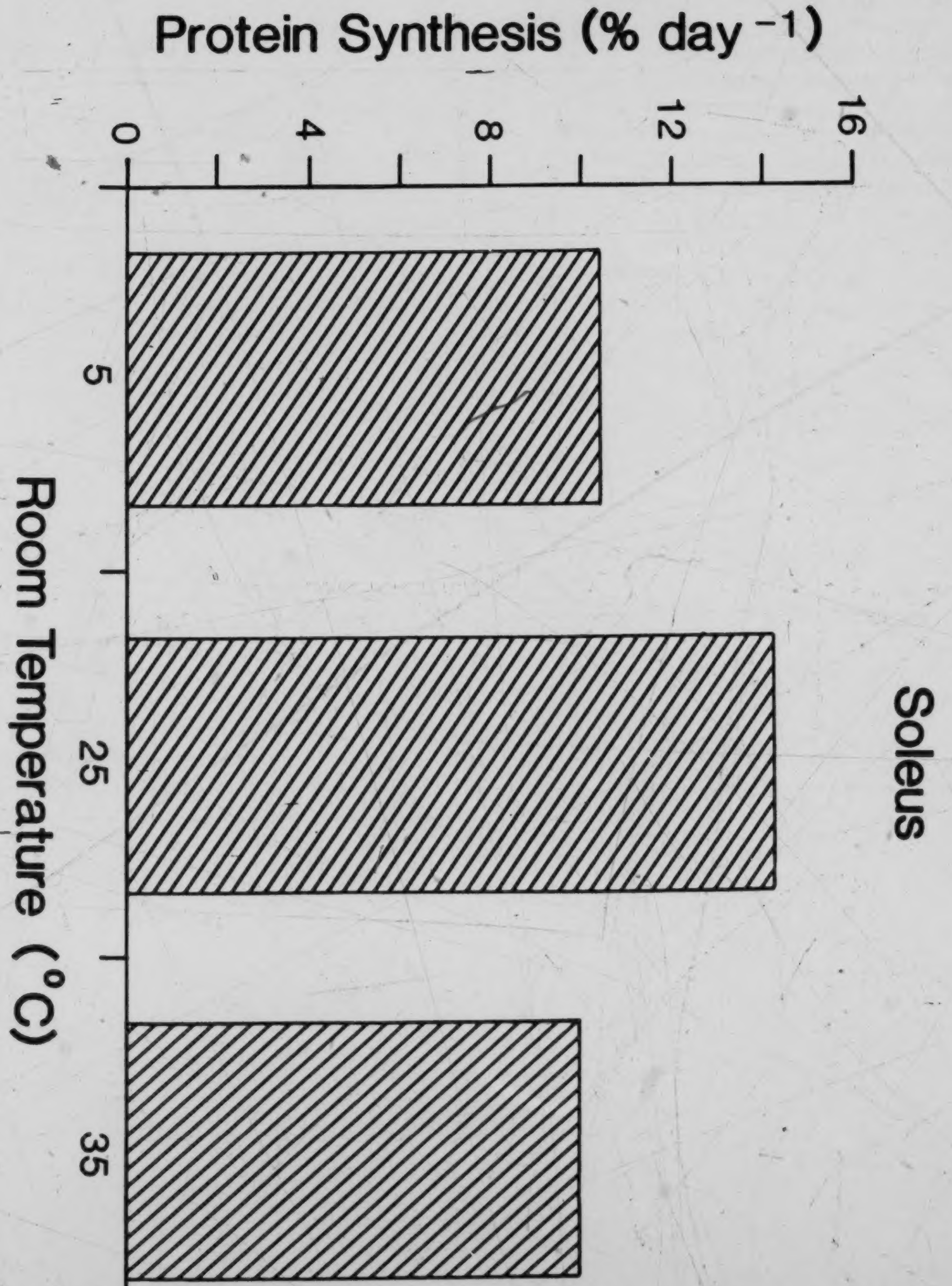

Soleus
Protein Synthesis (% day $^{-1}$)
0
4
8
12
16
5
25
35
Room Temperature (°C)

Figure A1-7. Fractional rate of protein synthesis (% day^{-1}) in the gastrocnemius muscle in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Mean values are from 9 to 10 rats.

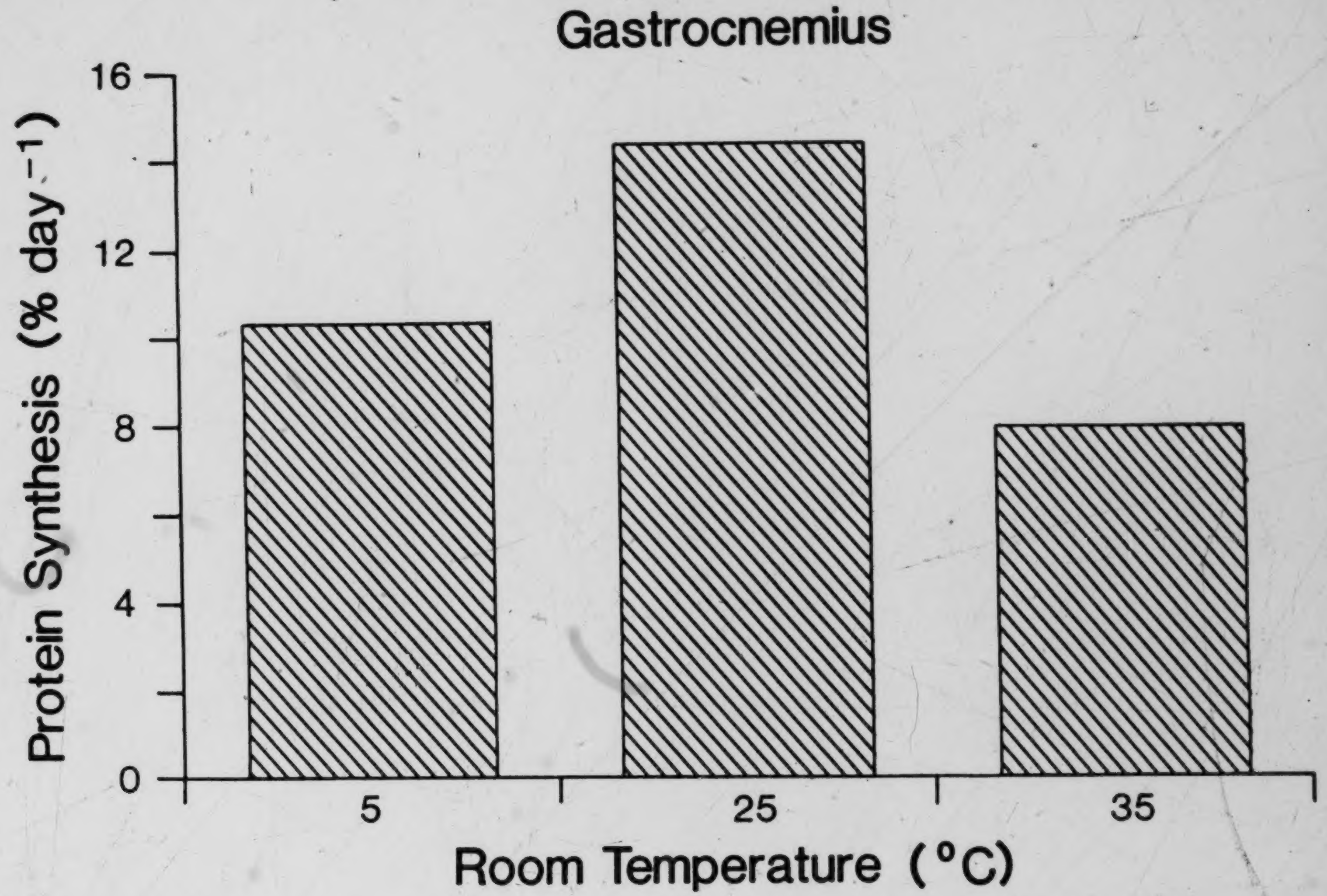
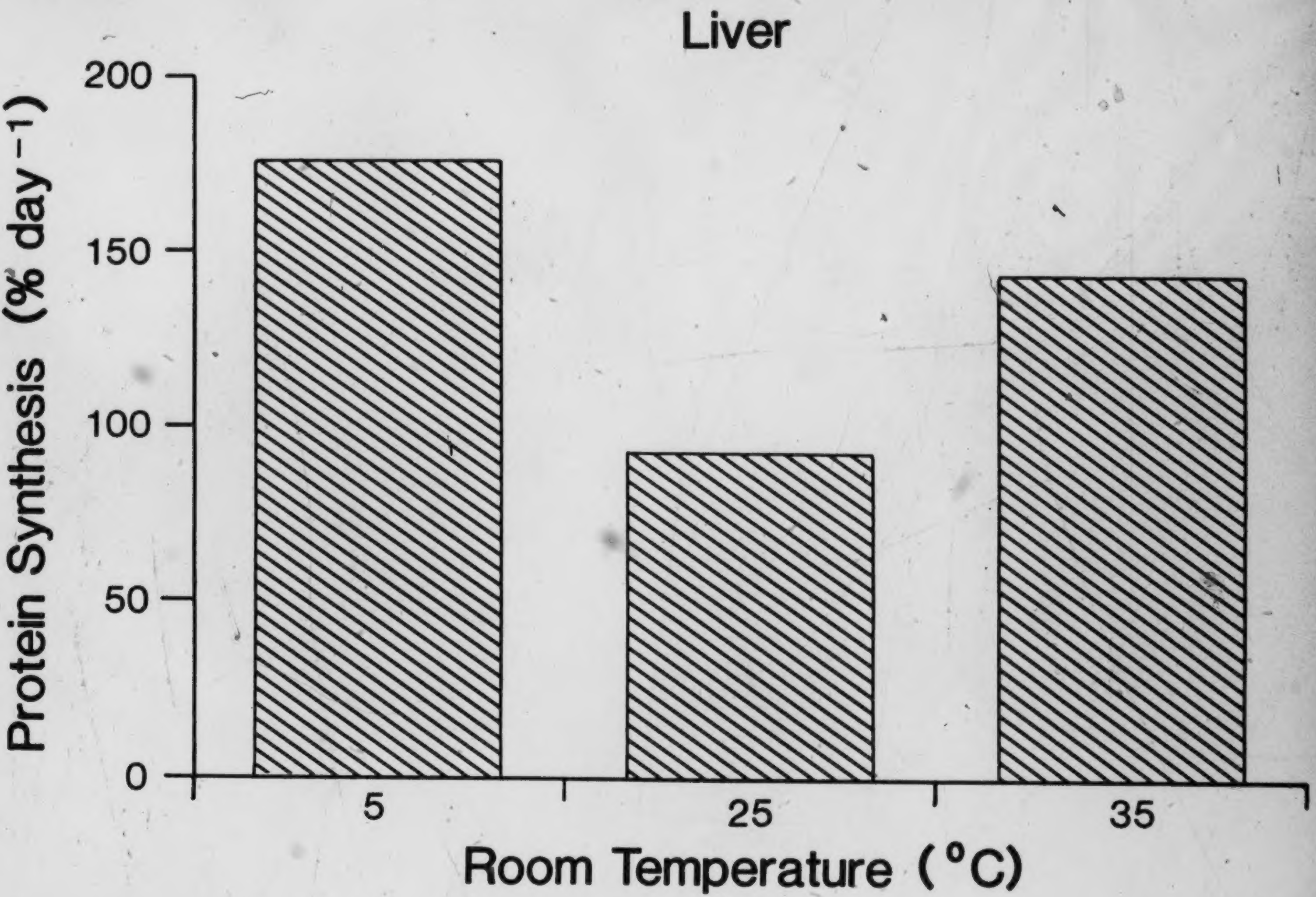

Gastrocnemius
Protein Synthesis (% day⁻¹)
16
12
8
4
0
5
25
35
Room Temperature (°C)

Figure A1-8. Fractional rate of protein synthesis (% day^{-1}) in the liver in rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Mean values are from 5 to 9 rats.


Liver
Protein Synthesis (% day -1)
200
150
100
50
0
5
25
35
Room Temperature (°C)

E. REFERENCES

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APPENDIX 2

TABLES CORRESPONDING TO THE FIGURES PRESENTED IN CHAPTER III

Table A2-1. Feed intake (g day^{-1}) of rats housed at three different temperatures cold (5 C), control (25 C) and hot (35 C) [@].

Time (days)	Group		
	Cold	Hot	Control
2	13.29 A	10.57 B	11.27 B
	(.24)	(.33)	(.30)
4	16.42 A	8.55 B	12.13 C
	(.29)	(.63)	(.44)
6	19.19 A	7.44 B	13.66 C
	(.35)	(.35)	(.53)
8	20.09 A	8.90 B	15.12 C
	(.42)	(.21)	(.39)
10	22.41 A	9.41 B	16.12 C
	(.35)	(.25)	(.54)
12	23.44 A	9.82 B	17.14 C
	(.73)	(.28)	(.60)
14	24.11 A	9.25 B	17.72 C
	(.62)	(.27)	(.56)
16	23.77 A	9.57 B	17.59 C
	(.93)	(.64)	(.90)
18	25.74 A	10.60 B	18.62 C
	(1.42)	(.35)	(1.03)
20	29.17 A	10.61 B	17.57 C
	(1.29)	(.35)	(2.94)
22	29.24 A	11.15 B	19.50 C
	(1.62)	(.50)	(.64)
24	29.11 A	11.28 B	20.58 C
	(1.80)	(.71)	(1.26)

[@] mean values from 6 to 30 rats as reported in Materials and Methods section.

( ) standard error of the mean

A,B,C-values in rows are different from each other at $P<0.05$.

Table A2-2. Mean body weights (g) of rats housed at three different temperatures cold (5 C) control (25 C) hot (35 C). Pair fed rats were housed at 25 C and fed an amount equivalent to the feed intake of rats housed at hot temperature.[a]

Group	Time (days)				
	5	10	15	20	25
Cold	97.83	140.85 AB	170.53 A	217.42 A	241.15 A
	(5.03)	(5.47)	(8.10)	(7.83)	(10.28)
Hot	103.82	123.17 A	159.97 A	176.68 B	192.18 B
	(5.82)	(7.00)	(3.97)	(6.72)	(4.02)
Control	110.98	154.92 B	190.72 B	248.75 C	275.78 C
	(10.00)	(8.98)	(6.39)	(13.69)	(10.66)
Pair Fed	100.70	117.38 A	142.02 C	160.12 B	179.35 B
	(9.84)	(5.49)	(2.60)	(5.35)	(2.76)

[a] mean values from 6 to 30 rats as reported in Materials and Methods section.

( ) standard error of the mean

A,B,C-values in columns are different from each other at $P<0.05$.

Table A2-3. Daily rate of bodyweight gain (g day $^{-1}$) in rats housed in cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the feed intake of rats housed at hot temperature.[a]

Time (days)	Group			
	Cold	Hot	Control	Pairfed
2	4.11 A	5.22 B	5.83 B	5.94 B
	(.14)	(.25)	(.21)	(.31)
4	4.08 A	3.18 B	6.21 C	3.89 B
	(.23)	(.32)	(.32)	(.24)
6	5.72 A	3.12 B	7.90 C	1.79 D
	(.25)	(.28)	(.29)	(.21)
8	6.25 A	5.26 B	8.03 C	3.55 D
	(.28)	(.20)	(.31)	(.76)
10	7.35 A	5.31 B	7.77 A	4.78 B
	(.27)	(.22)	(.29)	(.21)
12	6.14 A	5.72 A	8.59 B	3.69 C
	(.26)	(.15)	(.37)	(.19)
14	6.10 A	3.15 B	7.32 C	3.26 B
	(.25)	(.23)	(.36)	(.16)
16	6.55 A	4.54 B	7.43 A	2.58 C
	(.56)	(.24)	(.41)	(.16)
18	6.48 A	5.57 AB	7.94 A	3.78 B
	(.99)	(.47)	(.57)	(.22)
20	6.99 AB	5.41 A	8.48 B	2.90 C
	(.88)	(.35)	(.68)	(.62)
22	5.80 A	5.62 A	8.47 B	4.87 A
	(.91)	(.38)	(.53)	(.40)
24	5.91 AB	3.88 A	7.62 B	5.73 AB
	(1.15)	(.30)	(.87)	(.81)

[a] mean values from 6 to 30 rats as reported in Materials and Methods section.

( ) standard error of the mean

A,B,C,D-values in rows are different from each other at $P<0.05$.

Table A2-4. Weight of tissues (mg) from rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature.[a]

Tissue		Time (days)				
		5	10	15	20	25
Extensor Digitorum Longus	Cold	74.22 A	118.10	145.68 A	210.08	195.75 A
		(5.38)	(7.90)	(7.13)	(11.66)	(8.16)
	Hot	103.90 B	126.68	160.67 AB	189.47	203.25 A
		(7.65)	(7.76)	(11.79)	(9.98)	(5.08)
	Control	105.05 B	138.67	178.42 B	220.90	242.78 B
		(3.58)	(6.36)	(9.94)	(16.76)	(9.21)
	Pair Fed	94.73 B	120.90	151.93 A	188.40	193.52 A
		(12.24)	(8.44)	(4.80)	(18.38)	(5.35)
Soleus	Cold	72.98 A	88.47 A	110.73 A	159.72	161.75 A
		(4.89)	(4.04)	(4.94)	(13.32)	(10.36)
	Hot	92.72 B	99.87 A	135.48 B	175.17	179.40 A
		(6.19)	(5.56)	(5.97)	(12.56)	(6.89)
	Control	86.92 AB	117.20 B	154.38 C	201.98	215.30 B
		(4.03)	(2.86)	(8.72)	(9.97)	(14.66)
	Pair Fed	83.75 AB	96.25 A	129.07 B	154.93	177.07 A
		(5.02)	(3.10)	(3.78)	(13.24)	(6.95)
Gastrocnemius	Cold	836.86 A	1166.83	1581.00 A	1981.83 A	2277.18 A
		(35.38)	(31.79)	(64.26)	(88.48)	(116.96)
	Hot	1019.72 B	1260.67	1712.50 A	2155.50 A	2198.52 A
		(47.10)	(67.38)	(27.89)	(134.24)	(39.77)
	Control	1032.00 B	1345.50	1901.57 B	2636.00 B	2990.60 B
		(38.23)	(111.95)	(85.00)	(100.43)	(135.00)
	Pair Fed	971.33 B	1174.00	1612.17 A	1922.00 A	2112.80 A
		(49.52)	(49.94)	(37.36)	(67.78)	(84.98)
Heart	Cold	614.03 A	810.83 A	981.33 A	1128.00 A	1335.57 A
		(36.12)	(18.94)	(47.28)	(53.63)	(78.91)
	Hot	463.03 B	482.83 B	582.83 B	586.17 B	737.88 B
		(16.22)	(28.25)	(17.29)	(11.89)	(29.15)
	Control	517.63 B	637.17 C	780.52 C	878.00 C	1021.60 C
		(11.80)	(36.12)	(40.52)	(50.40)	(42.89)
	Pair Fed	463.72 B	446.17 B	522.33 B	511.83 B	636.13 B
		(15.35)	(15.07)	(10.57)	(16.34)	(18.27)
Liver	Cold	5550.0 A	8533.3 A	9450.0 A	13416.7 A	15000.0 A
		(356.6)	(388.7)	(649.5)	(1030.7)	(1075.8)
	Hot	4850.0 A	5466.7 B	7250.0 B	7150.0 B	8416.7 B
		(337.4)	(349.9)	(172.7)	(207.8)	(1200.5)
	Control	5750.0 A	8250.0 A	9883.3 A	13816.7 A	15316.7 A
		(331.4)	(549.4)	(502.3)	(1079.0)	(1041.6)
	Pair Fed	3650.0 B	4133.3 C	5250.0 C	5550.0 B	6583.3 B
		(156.5)	(321.1)	(283.7)	(254.8)	(307.0)

[a] mean values from 5-6 rats

( ) standard error of the mean

A,B,C-values in columns are different from each other at $P<0.05$.

Table A2-5. Weight of tissues (mg 100 g body weight^{-1}) adjusted for body weight from rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature.[a]

Tissue		Time (days)				
		5	10	15	20	25
Extensor Digitorum Longus	Cold	75.72 A	83.53 A	85.68 AC	96.50 A	81.23 A
		(3.16)	(3.21)	(3.22)	(2.89)	(1.42)
	Hot	97.20 B	102.90 B	100.43 B	112.73 B	105.83 B
		(4.23)	(2.61)	(1.54)	(2.78)	(2.61)
	Control	95.02 B	89.88 A	93.42 C	89.70 A	88.30 C
		(3.59)	(1.45)	(4.38)	(7.12)	(2.94)
	Pair Fed	93.92 B	102.90 B	106.93 B	117.25 B	107.87 B
		(2.34)	(1.58)	(2.30)	(2.74)	(2.09)
Soleus	Cold	74.02 A	62.83 A	65.20 A	73.45 A	66.92 A
		(3.39)	(1.75)	(2.58)	(5.48)	(2.55)
	Hot	89.63 B	79.87 B	84.55 BC	99.43 B	93.27 B
		(4.99)	(3.57)	(2.21)	(5.25)	(5.81)
	Control	78.42 AB	75.65 B	80.72 B	81.53 AB	77.78 C
		(3.00)	(2.32)	(2.82)	(2.48)	(3.16)
	Pair Fed	83.17 AB	82.42 B	90.85 C	96.25 B	98.77 B
		(3.91)	(2.69)	(1.82)	(6.09)	(3.97)
Gastrocnemius	Cold	868.68	832.07 A	930.12 A	917.52 A	944.73 A
		(12.61)	(25.20)	(26.59)	(52.85)	(29.93)
	Hot	988.42	1028.23 B	1073.17 B	1214.65 B	1147.32 B
		(34.68)	(36.13)	(26.15)	(33.11)	(37.03)
	Control	931.88	865.68 A	996.37 A	1065.73 C	1084.45 B
		(27.88)	(40.26)	(26.41)	(24.86)	(24.12)
	Pair Fed	962.97	1006.02 B	1135.07 B	1200.88 B	1176.38 B
		(18.87)	(42.09)	(13.71)	(20.56)	(34.44)
Heart	Cold	626.92 A	578.25 A	576.37 A	519.95 A	559.02 A
		(12.72)	(15.88)	(16.72)	(21.30)	(42.27)
	Hot	450.55 B	392.22 B	364.98 B	333.00 B	383.80 B
		(20.52)	(7.20)	(11.04)	(7.00)	(12.74)
	Control	467.77 B	412.58 B	398.53 B	353.28 B	370.77 B
		(8.99)	(13.30)	(14.92)	(7.02)	(10.01)
	Pair Fed	461.65 B	318.50 B	368.42 B	320.30 B	355.02 B
		(10.93)	(8.48)	(10.12)	(8.31)	(5.12)
Liver	Cold	5650.0 A	6066.7 A	5533.3 A	6150.0 A	6166.7 A
		(199.6)	(170.6)	(170.5)	(335.4)	(226.1)
	Hot	4650.0 B	4416.7 B	4533.3 B	4083.3 B	4383.3 B
		(143.2)	(116.7)	(135.8)	(185.1)	(175.9)
	Control	5166.7 AB	5333.3 C	5166.7 A	5500.0 A	5516.7 C
		(192.6)	(80.3)	(172.6)	(220.6)	(183.3)
	Pair Fed	3650.0 C	3516.7 D	3683.3 C	3483.3 B	3666.7 D
		(226.2)	(124.9)	(144.7)	(124.9)	(172.6)

[a] mean values from 5-6 rats

( ) standard error of the mean

A,B,C,D-values in columns are different from each other at $P<0.05$.

Table A2-6. Protein content (mg) in the muscles, heart and liver from rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature.[a]

Tissue		Time (days)			
		5	10	15	20
Extensor Digitorum Longus	Cold	13.2	20.1	23.0	30.4
		(.95)	(2.3)	(1.2)	(2.0)
	Control	16.7	18.6	27.5	33.8
		(1.6)	(.68)	(2.3)	(3.0)
	Hot	16.6	21.2	26.3	30.7
		(1.7)	(1.8)	(2.8)	(1.9)
	Pair Fed	17.2	16.5	22.7	23.9
		(2.0)	(1.3)	(1.9)	(3.3)
Soleus	Cold	11.7	13.9	18.2	21.7 A
		(.75)	(1.2)	(.99)	(.56)
	Control	13.2	17.4	24.3	30.6 B
		(.88)	(1.3)	(2.8)	(2.0)
	Hot	14.2	15.4	21.1	28.5 B
		(.85)	(1.0)	(1.0)	(2.2)
	Pair Fed	12.9	15.6	20.8	21.1 A
		(1.2)	(.19)	(1.2)	(2.0)
Gastrocnemius	Cold	132.2 A	186.9	246.7	260.3 B
		(3.9)	(9.6)	(9.9)	(6.0)
	Control	165.7 B	207.0	268.9	350.4 A
		(5.9)	(13.3)	(15.6)	(27.1)
	Hot	151.7	184.5	253.7	300.2 B
		(5.5)	(11.0)	(4.5)	(15.2)
	Pair Fed	158.9	171.2	252.7	278.5 B
		(11.3)	(8.3)	(11.1)	(11.4)
Heart	Cold	97.9 A	127.7 A	151.1 A	175.5 A
		(3.5)	(4.5)	(6.5)	(4.9)
	Control	78.2 B	100.2 B	112.5 B	141.5 B
		(4.6)	(9.5)	(8.6)	(8.7)
	Hot	73.6 A	82.0 A	102.4 A	98.8 C
		2.2	(4.9)	(3.9)	(4.5)
	Pair Fed	75.3 A	83.7 A	93.4 A	84.3 C
		(3.3)	(2.9)	(3.9)	(4.0)
Liver	Cold	730.9	1233.9 A	1333.5 A	1958.3 A
		(71.0)	(56.6)	(137.9)	(215.4)
	Control	800.1 A	1150.7 A	1364.0 A	1955.9 A
		(89.7)	(172.5)	(91.3)	(258.9)
	Hot	574.9	717.2 B	980.7 B	925.7 B
		(37.3)	(77.6)	(74.3)	(72.6)
	Pair Fed	530.5 B	633.5 B	783.1 B	732.0 B
		(46.3)	(24.6)	(64.7)	(32.5)

[a] mean values from 3-6 rats

( ) standard error of the mean

A,B,C,-values in columns are different from each other at P<0.05.

Table A2-7. Absolute rate of protein synthesis (mg day^{-1}) in the muscles, heart and liver from rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature.[a]

Tissue		Time (days)			
		5	10	15	20
Extensor	Cold	1.04	1.40	1.74	1.76
Digitorum Longus		(.26)	(.30)	(.42)	(.20)
	Control	1.60	1.68	2.45	2.00
		(.12)	(.13)	(.33)	(.32)
	Hot	1.28	1.27	1.42	2.03
		(.23)	(.22)	(.40)	(.20)
	Pair Fed	1.55	1.44	1.80	1.64
		(.16)	(.11)	(.64)	(.45)
Soleus	Cold	1.04	1.40	1.74	1.76
		(.10)	(.13)	(.17)	(.34)
	Control	1.60	1.68	2.45	2.00
		(.12)	(.12)	(.33)	(.32)
	Hot	1.28	1.27	1.76	2.03
		(.23)	(.09)	(.27)	(.48)
	Pair Fed	1.55	1.44	1.80	1.21
		(.16)	(.11)	(.26)	(.18)
Gastrocnemius	Cold	10.96	26.65	29.10	32.01 AB
		(2.07)	(4.10)	(4.33)	(3.69)
	Control	19.18	30.06	35.69	46.66 A
		(3.17)	(2.95)	(4.07)	(6.89)
	Hot	14.25	19.61	25.87	24.92 B
		(2.02)	(1.71)	(5.10)	(2.78)
	Pair Fed	14.85	20.81	27.13	23.69 B
		(1.16)	(3.17)	(3.45)	(3.40)
Heart	Cold	11.72	19.64 A	14.62 A	24.38 A
		(1.56)	(4.82)	(2.31)	(4.60)
	Control	10.67	9.24 B	11.88 AB	13.24 B
		(1.97)	(.80)	(4.80)	(2.96)
	Hot	6.40	5.81 B	7.04 BC	7.56 B
		(.68)	(.36)	(.64)	(1.21)
	Pair Fed	7.41	5.22 B	6.13 C	4.70 B
		(1.29)	(.30)	(.52)	(.26)
Liver	Cold	848.40	2199.95	3337.43	3564.25 A
		(258.69)	(431.38)	(1254.15)	(698.10)
	Control	983.75	1728.28	2049.03	3073.62 AB
		(452.08)	(914.05)	(312.93)	(211.27)
	Hot	841.47	1430.86	1914.25	1271.89 B
		(120.22)	(443.69)	(439.96)	(93.15)
	Pair Fed	780.36	1538.83	1176.64	961.13 AB
		(43.16)	(488.09)	(149.13)	(226.32)

[a] mean values from 3-6 rats

( ) standard error of the mean

A,B,C,-values in columns are different from each other at $P<0.05$.

Table A2-8. Absolute rate of protein degradation (mg day^{-1}) in the muscles, heart and liver from rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature.[a]

Tissue		Time (days)			
		5	10	15	20
Extensor Digitorum Longus	Cold	0.14	0.50	0.84	0.86
	Control	0.50	0.58	1.35	0.90
	Hot	0.36	0.35	0.50	1.11
	Pair Fed	0.85	0.74	1.10	0.94
Soleus	Cold	0.44	0.80	1.14	1.16
	Control	0.64	0.72	1.49	1.04
	Hot	0.43	0.42	0.91	1.18
	Pair Fed	0.74	0.63	0.99	0.41
Gastrocnemius	Cold	2.90	18.59	21.03	23.95
	Control	8.26	19.14	24.77	35.74
	Hot	6.15	11.51	17.77	16.82
	Pair Fed	7.39	13.35	19.67	16.23
Heart	Cold	6.76	14.68	9.67	19.42
	Control	7.12	5.69	6.33	9.69
	Hot	4.56	3.97	5.20	5.72
	Pair Fed	6.27	4.09	4.99	3.56
Liver	Cold	830.82	2132.38	3269.86	3496.68
	Control	924.18	1668.71	1969.46	3014.05
	Hot	616.90	1406.29	1889.68	1247.42
	Pair Fed	766.62	1525.09	1162.90	947.39

[a] mean values from 3-6 rats

Table A2-9. Plasma tri-iodothyronine concentration (n mol L^{-1}) from rats housed at cold (5 C), control (25 C) and hot (35 C) environmental temperatures. Pair fed rats were housed at 25 C and fed an amount equivalent to the previous days feed intake of rats housed at the hot temperature.[a]

Group	Time (days)				
	5	10	15	20	25
Cold	3.20 A	3.13 A	2.66	2.35	3.06 A
	(.51)	(.45)	(.69)	(.63)	(.29)
Hot	1.50 B	1.23 B	1.32	1.00	0.96 B
	(.19)	(.13)	(.53)	(.10)	(.14)
Control	2.05 B	2.20 AB	1.56	1.28	1.76 C
	(.27)	(.50)	(.10)	(.32)	(.16)
Pair Fed	1.76 B	1.79 AB	1.61	1.26	0.98 B
	(.22)	(.32)	(.15)	(.28)	(.13)

[a] mean values from 5-6 rats

( ) standard error of the mean

A,B,C,-values in columns are different from each other at $P<0.05$.